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Molecular basis of hematology

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The online version of this chapter contains an educational multimedia component on normal hematopoiesis.

Basic concepts

Advances in recombinant DNA technology over the past several decades have substantially altered our view of biologic processes and have immediate relevance to our understanding of both normal hematopoietic cell function and hematologic pathology. A complete review of molecular genetics is beyond the scope of this chapter, but it is intended as a review of the concepts of the molecular biology of the gene, an introduction to **epigenetics** and **genomics**, an outline of noncoding RNAs, concepts relevant for immunotherapeutic treatment approaches, and an explanation of the terminology necessary for understanding the role of molecular biology in breakthrough discoveries. Emerging diagnostic and therapeutic approaches in hematology are reviewed. The concepts outlined in the following sections also are illustrated in Figure 1-1; in addition, boldface terms in the text are summarized in the glossary at the end of this chapter. Several examples of how these concepts and techniques are applied in clinical practice are included.

Anatomy of the gene

Structure of DNA

DNA is a complex, double-stranded molecule composed of **nucleotides**. Each nucleotide consists of a **purine** (adenine or guanine) or **pyrimidine** (thymine or cytosine) base attached to a deoxyribose sugar residue. Each strand of DNA is a succession of nucleotides linked through phosphodiester bonds between the 5' position of the deoxyribose of one nucleotide and the 3' position of the sugar moiety of the adjacent nucleotide. The two strands are connected through hydrogen bonds between strict pairs of purines and pyrimidines; that is, adenine must be paired with thymine (A-T) and guanine must be paired with cytosine (G-C). This is known as Watson-Crick base pairing. Consequently, the two strands of DNA are said to be **complementary**, in that the sequence of one strand determines the sequence of the other through the demands of strict base pairing. The two strands are joined in an antiparallel manner so that the 5' end of one strand is joined with the 3' end of the complementary strand. The strand containing the codons for amino acid sequences is designated as the sense strand, whereas the opposite strand that is transcribed into messenger RNA (mRNA) is referred to as the antisense strand.

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Dr. Gruber declares no competing financial interest. Dr. Abdel-Wahab declares no competing financial interest.

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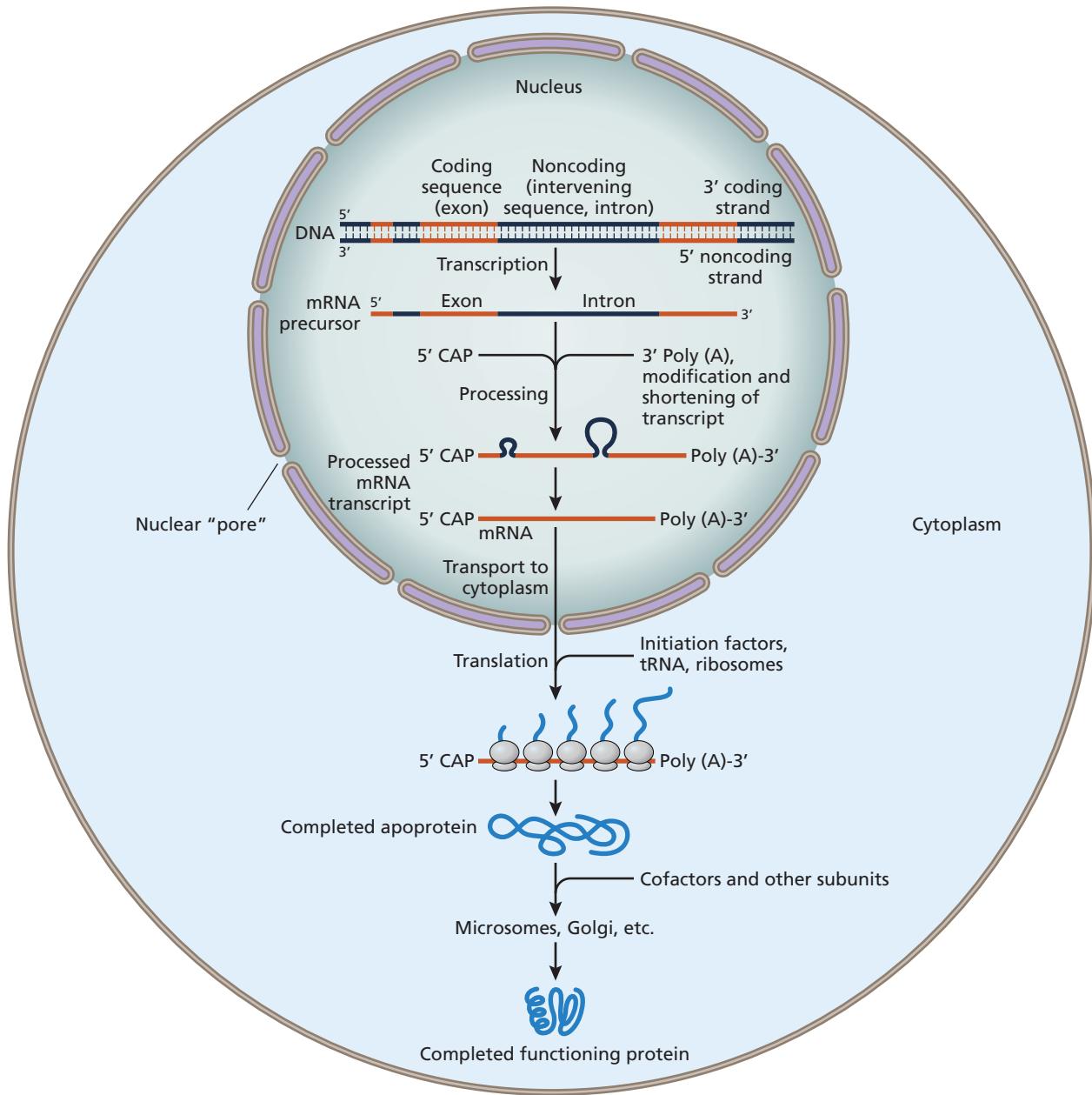


Figure 1-1 Flow of genetic information from DNA to RNA to protein. DNA is shown as a double-stranded array of alternating exons (red) and introns (pink). Transcription, posttranscriptional processing by splicing, polyadenylation, and capping are described in the text. The mature transcript passes from the nucleus to the cytoplasm, where it is translated and further modified to form a mature protein. Reproduced with permission from Hoffman R et al, eds., Hematology: Basic Principles and Practice, 6th ed. (Philadelphia, PA: Saunders Elsevier, Inc; 2013:5).

Structure of the gene

DNA dictates the biologic functions of the organism by the flow of genetic information from DNA to RNA to protein. The functional genetic unit responsible for the production of a given protein, including the elements that control

the timing and the level of its expression, is termed a **gene**. The gene contains several critical components that determine both the amino acid structure of the protein it encodes and the mechanisms by which the production of that protein may be controlled. The **coding sequence**, which dictates protein sequence, is contained within

exons; these stretches of DNA may be interrupted by intervening **noncoding sequences**, or **introns**. In addition, there are **flanking sequences** in the 5' and 3' ends of the coding sequences that often contain important regulatory elements that control the expression of the gene.

Genes are arrayed in a linear fashion along **chromosomes**, which are long DNA structures complexed with protein. Within chromosomes, DNA is bound in **chromatin**, a complex of DNA with histone and non-histone proteins that “shield” the DNA from the proteins that activate gene expression. The ends of chromosomes are capped by complexes of repetitive DNA sequences and associated proteins known as **telomeres**. The DNA replication machinery cannot effectively replicate the very ends of chromosomes, and thus shortening occurs with each cell division, ultimately leading to chromosomal instability and cellular senescence. Telomeres are therefore critical to protecting chromosome ends from degradation and fusion, and mutations in genes encoding components of the telomere complex are associated with the bone marrow failure syndrome dyskeratosis congenita. Furthermore, inappropriate activation of the enzymes that maintain telomeres (“telomerase”) is associated with numerous cancers, including hematologic malignancies.

Flow of genetic information

Transcription

RNAs are mostly single-stranded molecules that differ from DNA in two ways: by a sugar backbone composed of ribose rather than deoxyribose, and by containing the pyrimidine uracil rather than thymine. The first step in the expression of protein from a gene is the synthesis of a **pre-messenger RNA** (pre-mRNA). The **transcription** of pre-mRNA is directed by **RNA polymerase II**, which in conjunction with other proteins generates an RNA copy of the DNA sense strand. The introns are then removed by a complex process called **mRNA splicing**. This process involves the recognition of specific sequences on either side of the intron which allow its excision in a precise manner that maintains the exon sequence. The mRNA may then undergo modifications at the 5' and 3' ends (**capping** and **polyadenylation**, respectively). Although RNA splicing is largely restricted to the nucleus, it also can occur in the cytoplasm of platelets and neutrophils activated by external stimuli.

Splicing of mRNA is a critical step in gene expression, with important implications for understanding hematologic disease. Splicing is controlled by the spliceosome, a large complex of proteins (100 to 300) and five small

nuclear ribonuclear proteins (snRNPs). mRNA splicing is an important mechanism for generating diversity in the proteins produced by a single gene. Some genes exhibit **alternative splicing**, a process by which certain exons are included in or excluded from the mature mRNA, depending on which splice sequences are used in the excision process. For example, this is the means by which some erythroid-specific proteins of heme synthesis (aminolevulinic acid synthase) and energy metabolism (pyruvate kinase) are generated, contrasting with the alternatively processed genes in the liver and other tissues. This permits functional diversity of the products of the same gene and is one of several determinants of tissue specificity of cellular proteins. Mutations in the sequences of either introns or exons can derange the splicing process by either creating or destroying a splice site so that the intron sequence is not removed or the exon sequence eliminated. If abnormal splicing results in a premature stop codon (nonsense mutation), then a surveillance pathway known as **nonsense-mediated decay** may result in degradation of the abnormal mRNA. This mechanism generally applies to stop codon mutations in the first one-third to one-half of the mRNA and works to prevent synthesis of mutant peptides. When mutations occur in the last one-third of the mRNA molecule, abnormal peptides may be produced.

Mutations affecting the spliceosome machinery are found in a variety of hematopoietic malignancies. For example, splicing factor mutations (SF3B1, U2AF1, and SRSF2) are found in approximately 10% of patients with chronic lymphocytic leukemia and 50% of patients with myelodysplastic syndrome (MDS), as well as related myeloid malignancies. These mutations have now been shown to alter splicing in a manner distinct from loss-of-function, but the connection between these changes in splicing and clonal hematopoietic disorders is not yet well understood.

Translation

The mature mRNA is transported from the nucleus to the cytoplasm, where it undergoes **translation** into protein. The mRNA is “read” in a linear fashion by **ribosomes**, which are structures composed of ribonucleoprotein that move along the mRNA and insert the appropriate amino acids, carried by **transfer RNAs (tRNAs)**, into the nascent protein. The amino acids are encoded by three base triplets called **codons**, the **genetic code**. The four bases can encode 64 possible codons; because there are only 20 amino acids used in protein sequences, more than one codon may encode the same amino acid. For this reason, the genetic code has been termed **degenerate**. An amino

acid may be encoded by more than one codon; however, any single codon encodes only one amino acid. The beginning of the coding sequence in mRNA is encoded by AUG codon that has variable translation initiation activity determined by the neighboring nucleotide sequences (Kozak sequence). In addition, there are three **termination codons** (UAA, UAG, and UGA) that signal the end of the protein sequence.

Single-base-pair alterations in the coding sequence of genes (point mutations) may have a range of effects on the resultant protein. Because the genetic code is degenerate, some single-base-pair changes may not alter the amino acid sequence, or they may change the amino acid sequence in a manner that has no effect on the overall function of the protein; these are predicted to be phenotypically silent mutations. In other cases, mutations may lead to a loss or gain of protein function, or may result in the acquisition of a new function (**missense mutation**). Sickle cell disease is an example of a single base-pair change, resulting in an amino acid alteration that critically changes the chemical characteristics of the globin molecule. Other mutations may change a codon to a termination codon, resulting in premature termination of the protein (**nonsense mutation**). Finally, single or multiple base-pair insertions or deletions can disrupt the reading frame of genes. These **frame-shift mutations** render the gene incapable of encoding normal protein. These latter two abnormalities account for some β -thalassemias and for polycythemia due to a gain of function in the erythropoietin receptor. Clinically important mutations also may occur in the noncoding region of genes, such as in the regulatory elements upstream of the initiation codon or within intronic splicing sites.

Control of gene expression

With the exception of lymphocytes (which undergo unique changes in the DNA encoding immunoglobulin or the T-cell receptor) and germ cells (which contain only half of the DNA of somatic cells), each nucleated cell in an individual has the same diploid DNA content. Consequently, biologic processes are critically dependent on **gene regulation**, the control of gene expression such that proteins are produced only at the appropriate time within the appropriate cells. Gene regulation is the result of a complex interplay of specific sequences within a gene locus, chromatin, and regulatory proteins (transcription factors) that interact with those sequences to increase or decrease the transcription from that gene.

DNA sequences that lie in proximity to and regulate the expression of genes, which encode protein, are termed **cis-acting regulatory elements**. Nearly all genes have a site for binding RNA polymerase II that is within the

first 50 bases 5' to the structural gene and is called the **promoter** region. Other sequences that regulate the level of transcription of the gene are located at less predictable distances from the structural gene. Such sequences may increase (**enhancers**) or decrease (**silencers**) expression. A special type of enhancer is the locus control region, which was first defined in the β -globin cluster of genes on chromosome 11. It is located approximately 50 kilobases (kb) upstream from the β -globin gene, controls all genes within the β -globin locus, and also has a strong tissue-specific activity (erythroid-specific).

Control of gene expression is exerted through the interaction of the *cis*-acting elements described previously with proteins that bind to those sequences. These nuclear DNA binding proteins are termed **trans-acting factors** or **transcription factors**. Most of these proteins have a DNA-binding domain that can bind directly to regulatory sequences within the gene locus; many of them contain common motifs, such as **zinc fingers** or **leucine zippers**, which are shared by many transcription factors. In addition, they frequently have unique domains that allow them to interact with other transcription factors. Thus, a complex pattern exists whereby the expression of different transcription factors, which may interact both with one another and with specific regions of DNA to increase or decrease transcription, determines the unique tissue and stage-specific expression of the genes within a given cell.

Epigenetics

For a gene to be expressed, chromatin must be unwound and the DNA made more accessible to regulatory proteins. This is controlled by epigenetic processes or modifications to the genome that regulate gene expression without altering the underlying nucleotide sequence. These changes may be modulated by environmental factors and may be heritable. Epigenetic modulation of gene expression was first recognized in studies of glucose-6-phosphate dehydrogenase (G6PD), a protein encoded by an X-linked gene. Ernest Beutler deduced the principle of random embryonic X chromosome inactivation from studies of G6PD deficiency. His observations and the studies of Mary Lyon and Susumu Ohno on the mechanism of dosage compensation in mammals led to an understanding of X chromosome inactivation in females. This was the first example of stochastic epigenetic silencing in humans, demonstrating that human females are mosaics of the activity of X chromosome-encoded genes. Using this principle in tumor tissue derived from females led to early demonstrations that neoplastic diseases are, for the most part, **clonal**. Two common forms of epigenetic changes are DNA methylation and histone modifications.

DNA methylation

In addition to being complexed with protein, DNA is modified by the addition of methyl groups to cytosine residues (resulting in 5-methylcytosine) through enzymes called DNA methyltransferases. **Methylation** normally occurs throughout the genome and is associated with alterations in gene expression and processes such as X-chromosome inactivation, imprinting, aging, and carcinogenesis. It is generally a marker of an inactive gene, and changes in gene expression often can be correlated with characteristic changes in the degree of methylation of the 5' regulatory sequences of the gene. DNA methylation can silence genes through two mechanisms. In the first, the methylation mark itself can impair binding of transcription factors to the gene. Second, methylated DNA can be bound by proteins known as methyl-CpG-binding domain proteins that recruit additional chromatin remodeling proteins to the locus, which then modify histones leading to the formation of compact, inactive chromatin termed heterochromatin. In contrast to 5' regulatory sequences where methylation is typically associated with gene silencing, methylation of intragenic cytosine residues often coincides with active transcription of the gene within which they lie. The function and mechanism of gene body methylation are not well understood.

Mendelian genetics is based on the principle that the phenotype is the same whether an **allele** is inherited from the mother or the father, but this does not always hold true. Some human genes are transcriptionally active on only one copy of a chromosome (such as the copy inherited from the father), whereas the other copy on the chromosome inherited from the mother is transcriptionally inactive. This mechanism of gene silencing is known as **imprinting**, and these transcriptionally silenced genes are said to be “imprinted.” When genes are imprinted, they are usually heavily methylated in contrast to the nonimprinted copy of the allele, which typically is not methylated. A classic example of imprinting is the inheritance of Prader-Willi and Angelman syndromes, which are associated with a 4-megabase (Mb) deletion of chromosome 15. This region contains the gene associated with Angelman syndrome, *UBE3A*, which encodes a ligase essential for ubiquitin-mediated protein degradation during brain development. This gene is imprinted on the paternal allele. In addition, the region contains multiple genes associated with Prader-Willi syndrome, which are imprinted on the maternal allele. Thus, maternal inheritance of a mutation or deletion in *UBE3A* removes the single active copy of the gene and results in Angelman syndrome, and paternal inheritance of deletions in this region remove the only

active copies of the Prader-Willi-associated genes and result in Prader-Willi syndrome.

As DNA methylation modulates gene activity, acquired changes in methylation patterns are thought to contribute to functional alterations in hematopoietic stem cells during aging. Hematopoietic stem cells display an age-related decline in function and a relative loss of lymphoid differentiation potential, and these changes are associated with site-specific changes in DNA methylation. Furthermore, aberrant methylation may contribute to cancer. For example, mutations in enzymes affecting DNA methylation, most notably the DNA methyltransferase DNMT3A, are common in acute myeloid leukemia (AML). Mutations in DNMT3A are found in approximately 20% of adult AML patients and are associated with poor outcomes. These mutations appear to be acquired early in the evolution of the disease, thus likely serving as initiators of the disease. Other enzymes affecting DNA methylation, including TET2 and IDH1/2, are also recurrently mutated in AML, further illustrating the role of aberrant methylation in the pathogenesis of this disease. Of note, oxidation of methylated cytosines (5-methylcytosine) by members of the ten-eleven translocation (TET) protein family results in the formation of 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine. While the role of these epigenetic marks is not clearly understood, their levels are often altered in cancers (including AML), suggesting that they may contribute to the pathogenesis of disease. Given the prominent role of abnormal methylation in hematologic malignancies, small molecule inhibitors of DNA methyltransferases (eg, 5-azacitidine, decitabine) are used in the treatment of disorders that are characterized by aberrant DNA methylation (eg, MDS, AML).

Histone modification

Histones are DNA packaging proteins that organize DNA into structural units called nucleosomes. Octamers of the core histones—H2A, H2B, H3, and H4—make up the nucleosome around which 147 bp of DNA is wrapped, and histone H1 binds the “linker” DNA between nucleosomes. Histones are subject to multiple modifications—including methylation, acetylation, ubiquitination, phosphorylation, and others. The particular combination of histone modifications at any given locus is thought to confer a “histone code,” regulating processes such as gene expression, chromosome condensation, and DNA repair. Like methylation, histone modifications regulate gene activity, and therefore disruptions of the normal pattern of these modifications can contribute to cancer and other diseases. For example, hypoacetylation of histones H3 and H4 is associated with silencing of the cell cycle regulator

p21^{WAF1}, a gene whose expression is reduced in multiple tumor types. Aberrant expression levels of histone deacetylases, the enzymes that remove acetyl groups from histone tails, are common in hematopoietic malignancies, and histone deacetylase inhibitors are being tested in a variety of these diseases. The first of these inhibitors, vorinostat, is used in the treatment of cutaneous T-cell lymphoma. In addition to altered expression levels of histone-modifying enzymes, several fusion genes associated with hematologic malignancies aberrantly recruit histone modifiers to target genes, resulting in altered transcription (eg, the association of MLL fusion proteins with the DOT1L enzyme that methylates histones and the histone acetyltransferase p300 with AML1-ETO). Targeting histone modifiers therapeutically, therefore, has potential utility for a variety of cancers.

Noncoding RNAs

It has been estimated that only approximately 1% to 2% of the genome encodes protein, but a much larger fraction is transcribed. This transcribed RNA that does not encode protein is referred to as noncoding RNA (ncRNA) and is grouped into an increasingly large number of different classes, including **microRNAs**, small nucleolar RNAs (snoRNAs), **small interfering RNAs (siRNAs)**, Piwi-interacting RNAs (piRNAs), long noncoding RNAs (lncRNAs), and many others. Although each of these classes of ncRNAs differs in size, biogenesis pathway, and specific function, they share a common ability to recognize target nucleotide sequences through complementarity and regulate gene expression. The most well-described class of ncRNAs are microRNAs (miRs), whose biogenesis pathway is illustrated in Figure 1-2. Following transcription, a portion of this RNA (the pri-microRNA) forms hairpin loops that are cleaved by the enzymes **Drosha** and **Dicer** into short 21- to 23-bp double-stranded RNAs. These short double-stranded RNAs contain both sense strands and antisense strands that correspond to coding sequences in mRNAs. These mature miRs then are incorporated into a larger complex known as an **RNA-induced silencing complex (RISC)**. The miR is then unwound in a strand-specific manner, and the single-stranded RNA locates mRNA targets by Watson-Crick base pairing. Gene silencing results from cleavage of the target mRNA (if there is complementarity at the scissile site) or translational inhibition (if there is a mismatch at the scissile site). This gene-silencing pathway is known as RNA interference. As mediators of gene expression, miRs and other ncRNAs are expressed in a tissue-specific manner and play important regulatory roles in development and differentiation. Accordingly, dysregulation or mutations in ncRNAs are associated with various diseases, including cancer. In

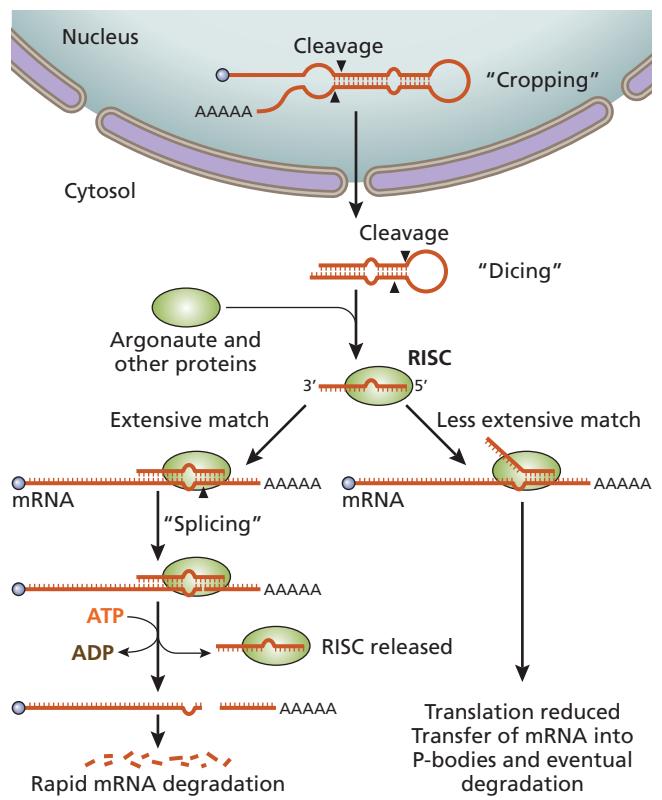


Figure 1-2 MicroRNA production. Production of microRNA begins with transcription of the microRNA gene to produce a stem-loop structure called a pri-microRNA. This molecule is processed by Drosha (“cropping”) to produce the shorter pre-microRNA. The pre-microRNA is exported from the nucleus; the cytoplasmic Dicer enzyme cleaves the pre-microRNA (“dicing”) to produce a double-stranded mature microRNA. The mature microRNA is transferred to RISC (RNA-induced silencing complex), where it is unwound by a helicase. Complementary base pairing between the microRNA and its target mRNA directs RISC to destroy the mRNA (if completely complementary) or halt translation (if a mismatch exists at the scissile site). Reproduced with permission from Alberts B et al, Molecular Biology of the Cell, 5th ed. (New York, NY: Garland Science; 2007).

hematopoiesis, numerous miRs that influence cell fate decisions have been identified. miR-223, for example, plays a central role in myeloid differentiation, and reduced expression of miR-223 is common in AML. Conversely, the expression of miR-125b, which is expressed in normal hematopoietic stem cells and whose targets include genes involved in regulating cell proliferation, differentiation, and survival, is increased in a variety of myeloid and lymphoid malignancies.

Molecular basis of neoplasia

Normal cellular growth and differentiation depends on the precise control of gene expression, and alterations in

the quantity or timing of gene expression can affect the survival and function of a cell. When such alterations occur in certain types of genes known as oncogenes or tumor suppressor genes, the cell may gain abnormal growth or survival properties, and accumulations of such mutations may lead to cancer.

Oncogenes

Oncogenes are genes that have the potential to cause cancer, and they arise from mutations in their normal counterparts termed *proto-oncogenes*. Proto-oncogenes generally code for proteins or ncRNAs that regulate such processes as proliferation and differentiation, and activating mutations or epigenetic modifications that increase the expression or enhance the function of these genes confers a growth or survival advantage on a cell. The first described oncogene, termed *SRC*, was discovered in the 1970s and is a member of a family of tyrosine kinases that regulate cell proliferation, motility, adhesion, survival, and differentiation. Activating mutations in the SRC family kinases are associated with the pathogenesis of multiple types of neoplasias; including cancers of the colon, breast, blood, head and neck, and others. Another classic example of an oncogene is the *BCR-ABL1* fusion gene found in chronic myelogenous leukemia (CML). This fusion results from a translocation between the *BCR* gene on chromosome 9 and the *ABL1* proto-oncogene on chromosome 22 and confers constitutive activation of *ABL1* and enhanced cell proliferation. Pharmacologic targeting of the activity of oncogenes, such as the use of the tyrosine kinase inhibitor imatinib to treat CML, can be an effective therapeutic approach.

Tumor suppressors

In contrast to oncogenes, **tumor suppressors** are genes that encode proteins or ncRNAs whose normal function is to inhibit tumor development through the promotion of such processes as apoptosis, DNA repair, cell cycle inhibition, cell adhesion, and others. Loss of the expression or function of these genes is associated with cancer, and generally both copies of the tumor suppressor gene must be altered to promote neoplasia. Thus, most tumor suppressors follow the “two-hit hypothesis” proposed by Alfred Knudson in his study of the retinoblastoma-associated tumor suppressor gene *RB1*. This gene encodes a protein that functions to regulate cell cycling and survival. Because both copies of the gene must be mutated for retinoblastoma to manifest, individuals that inherit a mutant allele (requiring just one more “hit” in the remaining normal allele for loss of gene function) generally develop disease earlier than those that must acquire “hits” in both alleles. Familial cancer syndromes often result from the inheri-

tance of heterozygous mutations in tumor suppressor genes. For example, Li-Fraumeni syndrome results from inherited mutations in the cell cycle regulator *TP53* and is associated with the early onset of multiple tumor types; including osteosarcoma, breast cancer, leukemia, and others. When mutations occur in the remaining normal allele, termed “loss of heterozygosity,” tumor growth is initiated. In some cases, loss of just one copy of a gene (“haploinsufficiency”) has been shown to contribute to cancer development. For example, loss of one copy of the ribosomal gene *RPS14* in patients with 5q- syndrome leads to aberrant ribosomal protein function and a block in erythroid differentiation.

Neoplasia and the immune system

The immune system defends and protects an individual through the detection of “nonself” antigens from either pathogens or infected/malignant cells, followed by expansion of effector cells that destroy them, as well as the development of immunological memory for subsequent defense. The ability of cancer to evade or escape the immune system is a hallmark of the disease and forms the basis of so-called immunotherapeutic approaches. These approaches include increasing the immunogenicity of cancer cells, as well as enhancing the immune response to the cells through a variety of mechanisms—including administration of cytokines, blocking negative regulators of T-cell function, and engineered cellular therapies. Two of the most successful approaches to date are **immune checkpoint inhibitors** and **chimeric antigen receptor T (CAR T) cells**, both of which enhance T-cell responses to tumor cells.

T cells initiate an immune response through the recognition of antigenic tumor peptides presented by the major histocompatibility complex (MHC) protein on the surface of either antigen-presenting or tumor cells by the T-cell receptor (TCR). TCR engagement alone is insufficient to activate T cells; they require an additional costimulatory signal by the CD80/B7 protein that engages with CD28 on T cells. In contrast, engagement of the CTLA-4 protein expressed on T cells by CD80/B7 leads to cell cycle arrest. This process modulates early steps in T-cell activation. During long-term antigen exposure, T cells upregulate PD-1, which inhibits T cells upon interaction with PD-L1 that is expressed on tumor cells. Thus CTLA-4 modulates early T-cell activation, whereas PD-1 functions in the effector phase. Immune checkpoint inhibitors that target both of these stages have been developed. Anti-CTLA-4 monoclonal antibodies modulate early steps in T-cell activation, whereas monoclonal antibodies directed against PD-1 or PD-L1 act to reverse inhibition of T cells that are present in the tumor microenvironment (Figure 1-3A).

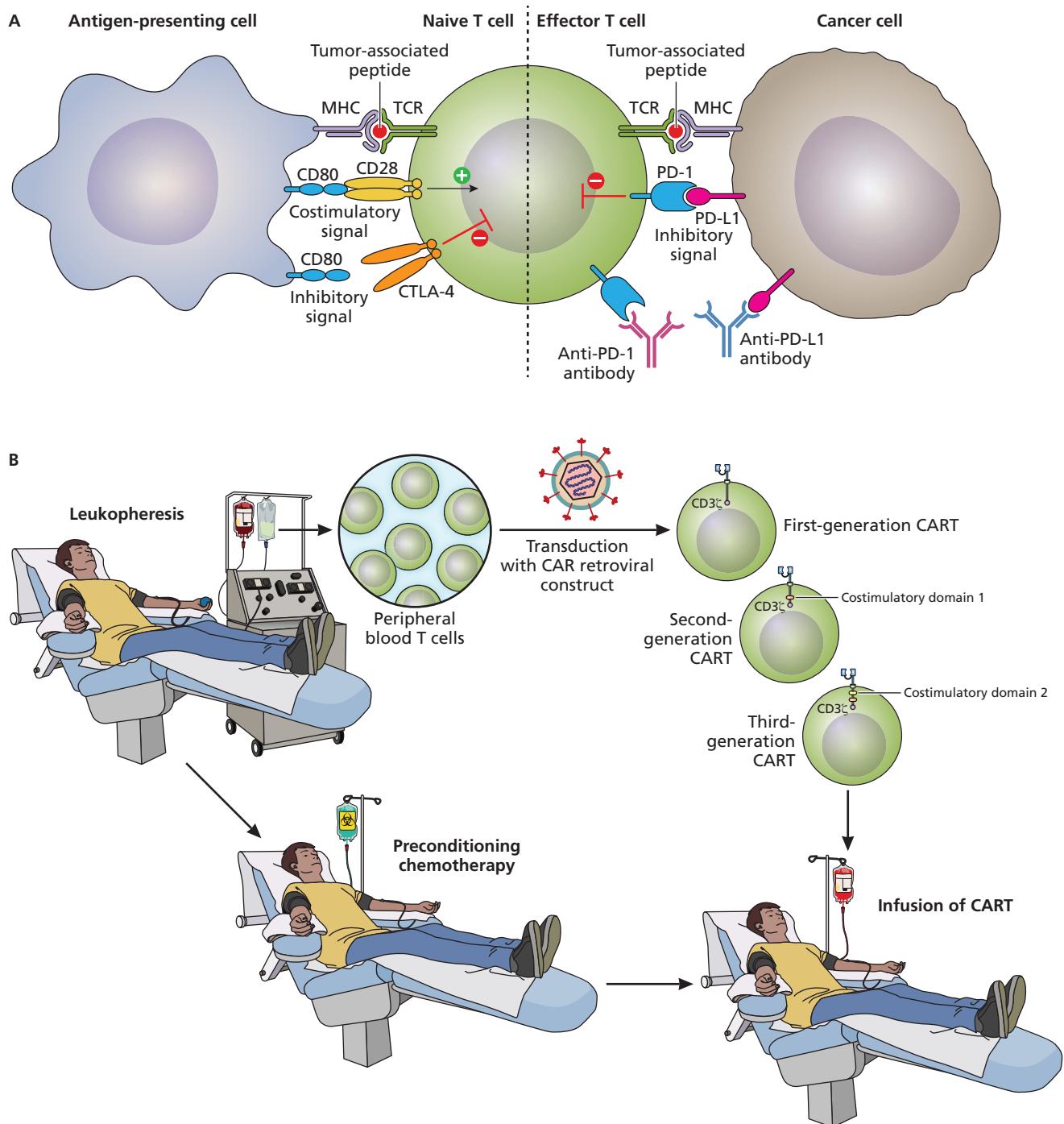


Figure 1-3 Immunotherapy approaches to malignancies. (A) Immune checkpoint inhibitors. Antigen-presenting cells and cancer cells present MHC-bound antigens to T cells. Recognition of the MHC-bound antigen by the TCR, in addition to CD80 engagement with CD28, leads to T-cell activation as indicated by the “+” symbol. In contrast, engagement of CD80 with the CTLA-4 protein leads to T-cell inhibition (“-” symbol). PD-L1 expression on cancer cells can associate with PD-1 on T cells, leading to inhibition in the T-cell effector phase. Antibodies to CTLA-4, PD-1, or PD-L1 block T-cell inhibition, enhancing the T-cell response to cancer cells. (B) CAR T-cell therapy. T cells are harvested from patients by pheresis followed by culture, transduction with a retrovirus carrying the genetic information to encode a chimeric antigen receptor (CAR), and expansion. Patients receive a preconditioning chemotherapy regimen that results in lymphodepletion prior to CAR T-cell infusion. This has been demonstrated to be beneficial for enhanced *in vivo* CAR T-cell expansion. The three generations of CARs are shown. First-generation CARs carry an extracellular domain that recognizes CD19 with an intracellular domain derived from the TCR (CD3 zeta). Second-generation CARs include a costimulatory domain to enhance T-cell activation upon engagement. Third-generation CARs carry two additional costimulatory domains.

As opposed to enhancing T-cell function by inhibiting negative regulators, CAR T-cell approaches isolate T cells from patients and modify them ex vivo with chimeric receptors that both target the cell to the tumor and then activate them upon target cell recognition (Figure 1-3B). Thus, recognition of a tumor cell by the patient's immune system is not necessary—the cells are removed and engineered for tumor cell recognition and effector function, followed by infusion back into the patient. The CAR is introduced into cells through infection with a lentivirus that carries the gene encoding the CAR, which consists of an extracellular antigen-recognition domain linked to an intracellular signaling domain. First-generation CARs had only the CD3 zeta intracellular domain of the T-cell receptor. Second- and third-generation CARs include additional costimulatory signaling domains that have resulted in enhanced persistence and proliferation once the cells were infused back into the patient. A variety of antigens have been evaluated for CAR T-cell therapy. CD19-directed cells have repeatedly demonstrated significant antitumor responses in patients with B-lineage acute lymphoblastic leukemia (ALL), leading to FDA approval for this indication in children and young adults.

While the clinical advances in anti-CD19 CAR T-cell therapy have demonstrated the clinical feasibility of this approach and provided evidence of the clinical importance of CAR T-cell therapy, a major challenge in the wider application of CAR T cells to other diseases is in the identification of appropriate antigens for CAR T cells. Anti-CD19 CAR T cells have been successful because CD19 is broadly expressed in B-cell malignancies and B-cell aplasia is tolerated. Ideally, however, CAR T cells should target a tumor-restricted antigen to avoid the toxicity that may result in an immune reaction against healthy tissues. In addition, the antigen should be broadly expressed on the majority of tumor cells and differentially expressed on tumor cells compared with essential normal tissue. Identifying such tumor-associated antigens for myeloid malignancies has been a significant challenge to date.

It is important to note that checkpoint inhibitors and CAR T cells have the capacity to elicit expected and unexpected toxicities. Anticipating and managing these toxicities are essential in the successful application of these therapies. For CAR T cells, the major toxicities encountered include: cytokine release syndrome, neurologic toxicity, “on-target/off-tumor” recognition, and anaphylaxis. Theoretical toxicities of off-target antigen recognition are possible and may be seen as further novel CAR T-cell antigens are studied in clinical trials. For immune checkpoint blockade, toxicities impacting the skin, gut, endocrine,

lung, and musculoskeletal systems are relatively common. The most common toxicities reported to affect each of these systems include rash, pruritus and/or vitiligo, diarrhea resulting from colitis, acute hypophysitis resulting in hypopituitarism, pneumonitis, and inflammatory arthritis. In contrast, cardiovascular, hematologic, renal, neurologic, and ophthalmologic toxicities occur much less frequently in the setting of checkpoint blockade. Consensus recommendations for the identification and management of cytokine release syndrome, as well as immune-related adverse events from checkpoint inhibitors, have been published by numerous groups.

Analytic techniques

Digestion, amplification, and separation of nucleic acids

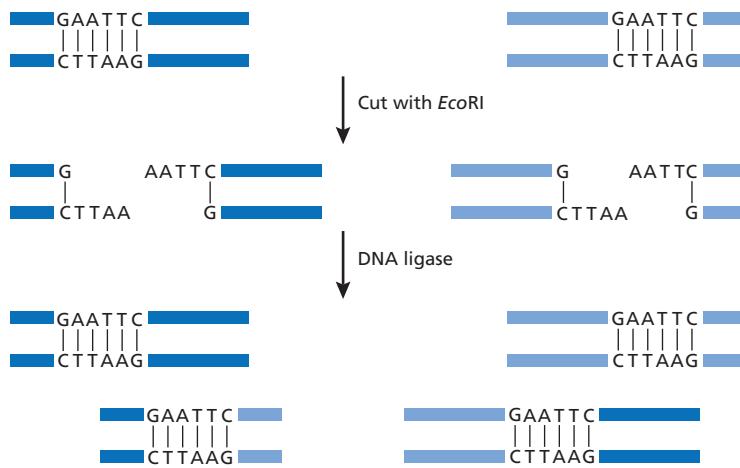
DNA may be cut, or digested, into predictable, small fragments using **restriction endonucleases**. Each of these bacterially derived enzymes recognizes a specific sequence of 4 to 8 bp in double-stranded DNA. These recognition sequences are usually palindromic (eg, they read the same sequence 5' to 3' on opposite strands). The DNA is cleaved by the enzyme on both strands at the site of the recognition sequence. After restriction endonuclease digestion, DNA fragments may be separated by size using agarose gel electrophoresis, with the smallest fragments running faster (closer to the bottom of the gel) and the largest fragments moving more slowly (closer to where the samples were loaded). DNA can be visualized in the gel by staining with either a fluorescent dye or the chemical ethidium bromide, both of which insert themselves between the DNA strands and fluoresce upon exposure to lasers and/or ultraviolet light. A desired fragment of DNA may be isolated and then purified from the gel. Some restriction enzymes generate overhanging single-stranded tails, known as “sticky ends.” Complementary overhanging segments may be used to join, or ligate, pieces of DNA to one another (Figure 1-4). These methods form the foundation of recombinant DNA technology.

Polymerase chain reaction

The polymerase chain reaction (PCR) is a powerful technique for amplifying small quantities of DNA of known sequence. Two **oligonucleotide** primers are required: one is complementary to a sequence on the 5' strand of the DNA to be amplified and the other is complementary to the 3' strand. The DNA template is denatured at high temperature; the temperature then is lowered for the prim-

A Restriction enzyme digestion

Enzyme	Recognition sequence	Digestion products	Overhang
EcoRI	5'--- GAATT C --- 3' 3'--- CTTAAG --- 5'	5'--- G 3' 5'AATT C --- 3' 3'--- CTTAA 5'	5'
SacI	5'--- GAGCT C --- 3' 3'--- CTCGAG --- 5'	5'--- GAGCT 3' 5'C --- 3' 3'--- C 5'	3'
PvuII	5'--- CAGCT G --- 3' 3'--- GTCGAC --- 5'	5'--- CAG 3' 5'CTG --- 3' 3'--- GTC 5'	Blunt

B Ligation of "sticky ends"**Figure 1-4** Restriction endonuclease digestion.

(A) Diagram of typical restriction enzyme recognition sequences and the pattern of cleavage seen upon digestion with that enzyme. (B) Means by which restriction enzyme can be exploited to form recombinant proteins. Digestion of the two fragments with the enzyme *Eco*RI results in four fragments. Ligation with DNA ligase can regenerate the original fragments but can also result in recombinant fragments in which the 5' end of one fragment is ligated to the 3' end of the second fragment. This recombinant DNA can then be used as a template for generation of recombinant protein in expression vectors.

ers to be annealed to the DNA. The DNA then is extended with a temperature-stable DNA polymerase (such as *Taq* polymerase), resulting in two identical copies of the original DNA from each piece of template DNA. The products are denatured, and the process is repeated. The primary product of this reaction is the fragment of DNA bound by the two primers. Thus, small quantities of input DNA may be used to synthesize large quantities of a specific DNA sequence. This technique has superseded many blotting techniques for prenatal diagnosis and cancer diagnostics. Using multiple primer pairs in the same reaction, multiplex PCR can efficiently amplify several fragments simultaneously.

Reverse transcriptase PCR (RT-PCR) is a modification of the PCR technique that allows the detection and amplification of expressed RNA transcripts. **Complementary DNA (cDNA)** is generated from RNA using reverse transcriptase, an enzyme that mediates the conversion of RNA to DNA. The resultant cDNA is then subjected to routine PCR amplification. Because cDNA is generated from processed mRNA transcripts, no intronic sequences are obtained. RNA is much less stable than DNA; thus, am-

plication of mRNA from tissue or blood requires careful preservation of source tissue or blood samples.

Quantitative PCR is another modification of the PCR technique. The most commonly used method is **real-time PCR**, in which a fluorogenic tag is incorporated into an oligonucleotide that will anneal to the internal sequence of the *Taq* DNA polymerase-generated PCR product. This tag consists of a fluorescent "reporter" and a "silencing" quencher dye at opposite ends of the oligonucleotide. When annealed to the internal sequence of the PCR product, fluorescence from the reporter is quenched because the silencer is in proximity. After completion of each cycle of PCR amplification, the reporter is not incorporated in the product but is cleaved by *Taq* DNA polymerase (because this enzyme also has exonuclease activity). This fluorogenic tag is released, generating a fluorescent signal (Figure 1-5). Real-time PCR detects the number of cycles when amplification of the product is exponential and expresses this as a ratio to standard housekeeping RNA, such as ribosomal RNA or glyceraldehyde-3-phosphate dehydrogenase mRNA. This number can be converted to the number of molecules of mRNA present in the test

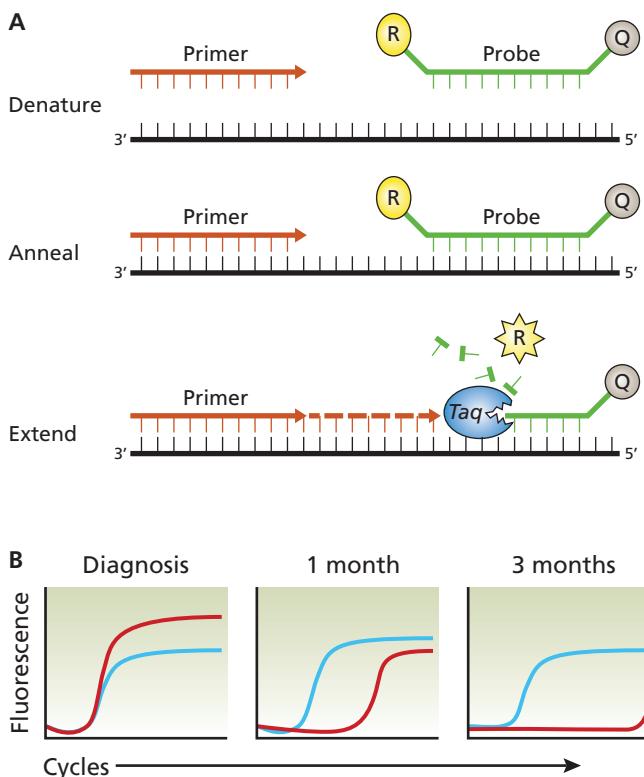


Figure 1-5 Real-time PCR. (A) Sample DNA (or cDNA) is denatured, and target-specific primers are annealed to begin the PCR amplification (shown for one strand). An oligonucleotide probe complementary to a sequence within the PCR product is included in the reaction. The probe contains a fluorophore (R) covalently attached to the 5' end and a quencher (Q) at the 3' end. As the *Taq* polymerase extends the nascent strand, its 5'-to-3' exonuclease activity degrades the probe, releasing the fluorophore from the quencher and allowing the fluorophore to fluoresce. An example of this fluorescent readout is shown in panel B, which depicts the relative fluorescence intensity from amplification of the *BCR-ABL* fusion transcript (yellow line) to an endogenous control transcript (green line) in a patient with chronic myelogenous leukemia before and after treatment with a tyrosine kinase inhibitor. The cycle number at which fluorescence crosses a threshold (horizontal dotted line) is inversely proportional to the amount of template DNA or cDNA. Although the control template is consistently detected throughout therapy, the *BCR-ABL* transcript abundance is lower at 1 month (higher cycle threshold) and undetectable at 3 months into therapy.

sample. This technique is used widely to measure minimal residual disease (MRD) or to monitor clearance of *BCR/ABL* transcripts in patients treated with tyrosine kinase inhibitors.

The power of PCR lies in its great sensitivity, but this is also a potential weakness because small amounts of contaminating DNA or RNA from other sources can cause false-positive results. Clinical laboratories that use PCR for critical diagnostic tests require elaborate quality assurance

protocols to prevent inappropriate diagnosis. Equally troublesome are false-negative results that result from inappropriate primer design, degraded RNA, or inappropriate temperature parameters for the annealing of primers.

The amplified sequence of interest then can be rapidly evaluated for presence of mutation(s) by direct sequencing, restriction enzyme digestion (if a suitable enzyme that discriminates between mutant and wild-type alleles is available), allele-specific PCR (discussed later in this chapter), or other techniques.

Hybridization techniques

DNA is chemically stable in its double-stranded form. This tendency of nucleic acids to assume a double-stranded structure is the basis for the technique of **nucleic acid hybridization**. If DNA is heated or chemically denatured, the hydrogen bonds are disrupted and the two strands separate. If the denatured DNA is then placed at a lower temperature in the absence of denaturing chemicals, the single-stranded species will reanneal in such a way that the complementary sequences are again matched and the hydrogen bonds re-form. If the denatured DNA is incubated with radioisotope- or fluorogen-labeled, single-stranded complementary DNA or RNA, the radiolabeled species will anneal to the denatured, unlabeled strands. This hybridization process can be used to determine the presence and abundance of an identical DNA species. The technique of molecular hybridization is the basis for Southern blotting and many other molecular techniques.

Southern blotting (Figure 1-6) is used to detect specific DNA sequences. In this procedure, electrophoretically separated DNA fragments are separated to a filter membrane and subsequent fragment detection by probe hybridization. **Restriction fragment-length polymorphism (RFLP)** analysis is a Southern blot-based technique with many useful applications in hematology. Using this technique, inherited disease-associated alleles may be identified and traced by the presence of inherited mutations or variations in a DNA sequence that create or abolish restriction sites. Rarely, a single-base, disease-causing DNA mutation will coincidentally fall within a recognition sequence for a restriction endonuclease. If a probe for the mutated fragment of DNA is hybridized to total cellular DNA digested with that enzyme, then the detected DNA fragment will be a different size. The β -globin point mutation resulting in hemoglobin S may be detected in this way. More commonly, genetic diseases are not the result of single base-pair mutations that conveniently abolish or create restriction enzyme sites. A similar technique may be used, however, to detect the presence of an RFLP that is linked to a disease locus within a family or group, but

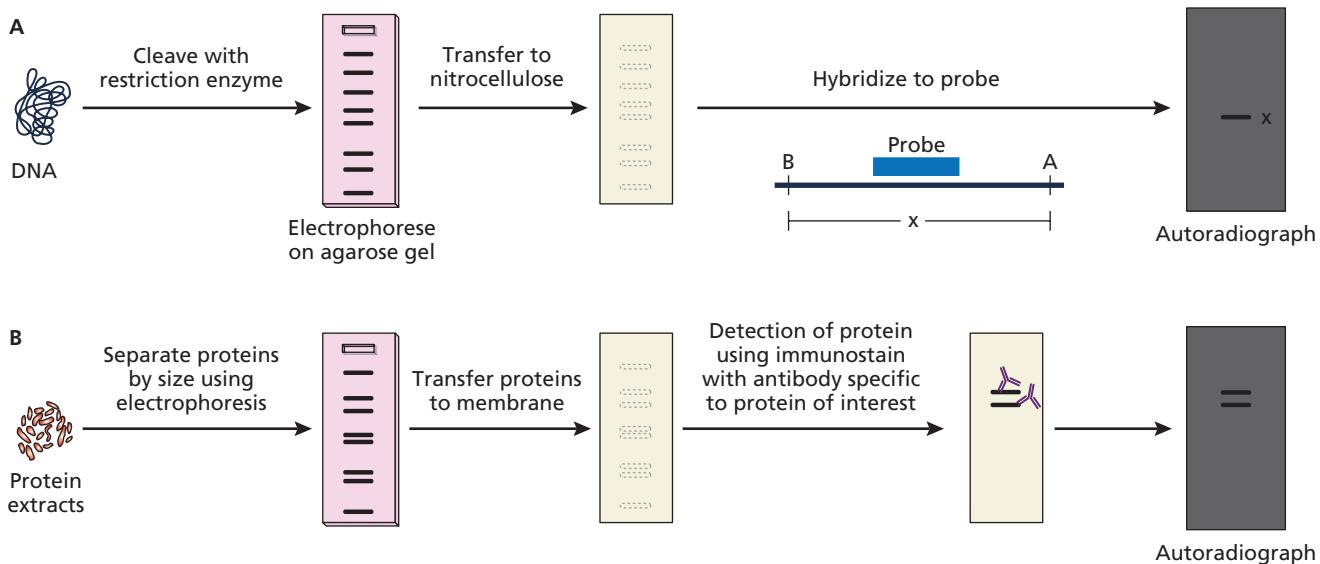


Figure 1-6 Common hybridization techniques in molecular biology. (A) Southern blot analysis of DNA. DNA is cleaved with a restriction endonuclease, electrophoresed through an agarose gel, and transferred to nitrocellulose. The probe, as illustrated at the bottom of the figure, lies on a piece of DNA of length x when DNA is digested with an enzyme that cleaves at sites A and B. Hybridization of the probe to the blot, with appropriate washes and exposure to radiograph, shows a single band of length x on the autoradiogram. (B) Western blot detection of protein. Proteins are extracted from cells and then separated by gel electrophoresis to separate proteins based on length of denatured polypeptide (or occasionally based on 3D structure of native proteins). The separated proteins are then transferred to a membrane where they are stained with antibodies to detect a protein of interest. Detection antibodies for Western blot are commonly conjugated to an enzyme which allows for detection of the protein of interest in the membrane using a variety of methodologies such as colorimetric or chemiluminescent detection.

that does not directly detect the molecular abnormality responsible for the disease. This is because there are normal variations in the DNA sequence among individuals that are inherited but silent in that they do not cause disease. These **polymorphisms** may be located in intronic sequences or near the gene of interest. They are surrogates that can be used to identify the region of DNA containing the genetic variant in question. Because RFLPs are transmitted from parent to offspring, they are extremely useful in the diagnosis of many genetic diseases.

Hybridization techniques also can be applied to RNA. Although RNA is generally an unstable single-stranded species, it is stabilized when converted to the double-stranded form. Therefore, if placed under hybridization conditions, RNA will complex with complementary, single-stranded nucleic acid species in the same fashion as DNA. **Northern blotting** is analogous to Southern blotting, but it involves electrophoresis of RNA with subsequent transfer and hybridization to a probe. Whereas Southern blotting detects the presence of a gene or its integrity, Northern blot analysis detects the level of expression of a gene within a specific cell type.

Protein can be detected by the blotting technique referred to as **Western blotting** (Figure 1-6). Proteins are detected by specific antibodies directed against the pro-

tein of interest. A labeled anti-immunoglobulin antibody raised in another species then can be used to detect the specific antibody bound to the blot.

Cytogenetic techniques

Uniform, nonrandom chromosomal abnormalities, termed *clonal abnormalities*, can be detected in malignant cellular populations by metaphase **cytogenetics**, or chromosomal analysis. Conventional cytogenetic techniques can detect numeric chromosomal abnormalities (too many or too few chromosomes), as well as deletion or translocation of relatively large chromosomal fragments among chromosomes. Certain chromosomal translocations are considered pathognomonic of specific diseases, such as the t(15;17) in acute promyelocytic leukemia. Normally, chromosomes cannot be seen with a light microscope, but during cell division they become condensed and can be analyzed. To collect cells with their chromosomes in this condensed state, bone marrow or tumor tissue may be briefly maintained in culture and then exposed to a mitotic inhibitor, which blocks formation of the spindle and arrests cell division at the metaphase stage. Thus, cytogenetic studies require dividing cells.

Conventional cytogenetic studies have several limitations. First, these studies require active cell division, which

may not be feasible for some clinical samples. Second, the technique is insensitive to submicroscopic abnormalities. Finally, because only a very small number of cells are analyzed, the technique is relatively insensitive for measurement of MRD burden.

Fluorescence in situ hybridization (FISH) studies complement conventional cytogenetic analysis by adding convenience, specificity, and sensitivity. This technique applies the principles of complementary DNA hybridization. A specific single-stranded DNA probe corresponding to a gene or chromosomal region of interest is labeled for fluorescent detection. One or more probes are then incubated with the fixed cellular sample and examined by fluorescence microscopy. FISH probes have been developed that can identify specific disease-defining translocations, such as the t(15;17) that characterizes acute promyelocytic leukemia. A probe corresponding to the *PML* gene on chromosome 15 is labeled with a fluorescent marker, such as rhodamine, which is red. Another fluorescent marker, such as fluorescein, which is green, is linked to a probe corresponding to the *RARA* gene on chromosome 17. When the t(15;17) chromosomal translocation is present, the two genes are juxtaposed, the two probes are in proximity, and the fluorescent signals merge to generate a yellow signal. The specificity of FISH is highly dependent on the probes that are used. Numeric abnormalities, such as monosomy and trisomy, may be identified using centromere-specific probes.

The major advantage of FISH is that it can analyze known cytogenetic abnormalities in nondividing cells (interphase nuclei); thus, peripheral blood slides can be directly processed. FISH studies are most useful when assessing for the presence of specific molecular abnormalities associated with a particular clinical syndrome or tumor type and are approximately 1 order of magnitude more sensitive than morphology and conventional cytogenetic studies in detecting residual disease. FISH panels are now available to detect recurrent genetic changes in leukemias, lymphomas, and multiple myeloma. These panels are particularly useful in predicting prognosis when conventional cytogenetic studies are noninformative.

Since their introduction nearly 30 years ago, FISH techniques have evolved rapidly for use in hematologic disorders. For example, double-fusion FISH (D-FISH) uses differentially labeled large probes that each span one of the two **translocation breakpoints**. This allows simultaneous visualization of both fusion products and reduces false-negative results. Another technique known as break-apart FISH uses differentially labeled probes targeting the regions flanking the breakpoint. Thus, in normal cells, the signals appear fused but they split upon translocation. This

technique has been used to detect *MYC* translocations in Burkitt lymphoma and *CCND1* translocations in mantle cell lymphoma. Labeling probes with unique combinations of fluorophores in multiplex FISH (M-FISH) not only has permitted simultaneous detection of every chromosome but also now has been used to analyze specific chromosomal regions and can detect subtle rearrangements.

Array-based techniques

DNA microarrays are composed of oligonucleotide probes spanning sites of known **single-nucleotide polymorphisms (SNPs)**. Fluorescently labeled single-stranded DNA from a test sample is hybridized on the array to determine, for a specific region in the genome, which DNA sequence undergoes complementary base pairing with the sample. The pattern of hybridization signals is analyzed using computer software, providing a detailed profile of genetic variation specific to an individual's DNA. With current technology, a single microarray has sufficient density to analyze variation at >1 million polymorphic sites. These data can be analyzed in several ways. First, the genotypes at each site can be used in genome-wide association studies in which the allele frequencies at each SNP are compared in disease cases and unaffected controls. Second, the intensity of fluorescent signals from multiple adjacent sites can be used to infer changes in the abundance of DNA across the genome. Changes in DNA content may include inherited **copy number variants** or somatically acquired deletions and amplifications present in tumor samples. Finally, long stretches of homozygosity that reflect acquired **partial uniparental disomy**, a recurrent abnormality present in a variety of myeloid malignancies, can be identified. In a variation of this technique, the relative abundance of methylated versus unmethylated DNA can be detected in samples by pretreating DNA with chemicals (eg, bisulfite) that convert methylated cytosine bases before hybridization on an array.

In **comparative genomic hybridization (CGH)**, DNA extracted from a test sample (eg, tumor) and a matched normal control (eg, buccal wash) is differentially labeled and hybridized to a microarray composed of oligonucleotide probes. The ratio of test to control fluorescence is quantified using digital image analysis. Similar to SNP arrays, amplifications in the test DNA are identified as regions of increased fluorescence ratio, and losses are identified as areas of decreased ratio. In array CGH, resolution of the analysis is restricted by probe size and the density of probes on the array. These and other techniques permit high-resolution, genome-wide detection of genomic copy number changes. Careful analysis of AML

genomes using these approaches has revealed few somatic copy number changes that are not detectable by routine cytogenetics. In contrast, ALL genomes are characterized by recurring copy number alterations, frequently involving the loss of the genes required for normal lymphoid development (eg, *PAX5*, *IKZF1*).

RNA expression arrays allow for comprehensive characterization of the gene expression patterns within the cells of interest, referred to as a **gene expression profile**. This technique has been used to classify disease, predict response to therapy, and dissect pathways of disease pathogenesis. To perform these assays, mRNA is extracted from samples, and double-stranded cDNA is synthesized from the RNA template. Then, biotinylated complementary RNA (cRNA) is generated from the cDNA template by in vitro transcription using biotin-labeled nucleotides. The biotinylated cRNA is fragmented and incubated with probes in a solution or hybridized to a microarray. Hybridization is then detected using a streptavidin–phycoerythrin stain, and the fluorescence intensity of each feature of the array is quantified.

Two main computational approaches have been used to analyze microarray data: unsupervised and supervised learning. Unsupervised learning methods cluster samples based on gene expression similarities without a priori knowledge of class labels. Hierarchical clustering and self-organizing maps are two commonly used algorithms of unsupervised learning. One potential application of unsupervised learning is for discovery of previously unrecognized disease subtypes. The strength of this method is that it provides an unbiased approach to identifying classes within a data set. A weakness is that these data sets are complex, and the structure uncovered by clustering may not reflect the underlying biology of interest. The second computational approach, supervised learning, uses known class labels to create a model for class prediction. For example, a training data set is used to create an expression profile for tumor samples from patients with “cured” versus “relapsed” disease. These profiles then are applied to an independent data set to validate the ability to make the prognostic distinction. In either method, it is important to demonstrate statistical significance and ensure that the tested samples are compared with the appropriate controls.

Once differentially expressed genes are defined, then it is also often helpful to next determine whether the differentially expressed genes that are identified belong to specific pathways of known biological significance. This is commonly done through the use of the GO (Gene Ontology) or KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathway analysis database. Also, a statistical methodology known as GSEA (gene set enrichment analysis) is also com-

monly used to determine if the differentially expressed genes are statistically enriched in previously published and defined gene sets publicly deposited in prior microarray or RNA-seq datasets.

The main limitation of microarray expression technology is that it analyzes only mRNA abundance. It does not reveal important translational and posttranslational modifications and protein–protein interactions. Purity of the cell population is also essential for these analyses, and one must ensure that the control and analyzed cells are homogeneous, of the same cell type, and at comparable stages of differentiation. It is advisable that any significant difference in mRNA expression detected using microarray technology be confirmed using an orthogonal approach (eg, real-time PCR).

Sequence-based studies

Analysis of DNA sequence variation by conventional techniques (eg, Sanger sequencing) is being replaced rapidly by a variety of novel high-throughput technologies (collectively termed **next-generation [next-gen] sequencing**). These developments have greatly accelerated the pace and lowered the cost of large-scale sequence production. At the core of each of these technologies is the preparation of DNA fragment libraries, which are then clonally amplified and sequenced by synthesis in multiple parallel reactions (Figure 1-7). Sequencing both ends of the DNA templates (“paired-end reads”) improves the efficiency of data production and facilitates the identification of insertions, deletions, and translocations. With these approaches, the search for inherited and somatic mutations associated with hematologic malignancies and congenital blood disorders has evolved from a candidate gene approach to unbiased surveys of all coding and noncoding regions of the genome.

The DNA libraries used for these sequencing reactions can be prepared from whole genomes or from selected regions of interest. For example, the regions of the genome that encode proteins (the **exome**) can be enriched by hybridizing DNA to oligonucleotide probes before library construction. Exome sequencing is preferred for many studies (compared with whole genome sequencing) because the cost of sequence production is lower and the interpretation of sequence variants in protein-coding genes is more tractable. A limitation of exome sequencing is that it cannot detect structural variants (such as deletions, amplifications, and rearrangements). In addition, the use of DNA from tissue uninvolved in the disease process is very important for whole exome or genome sequencing to identify potential somatic alterations specific for the disease tissue, given the large number of sequence

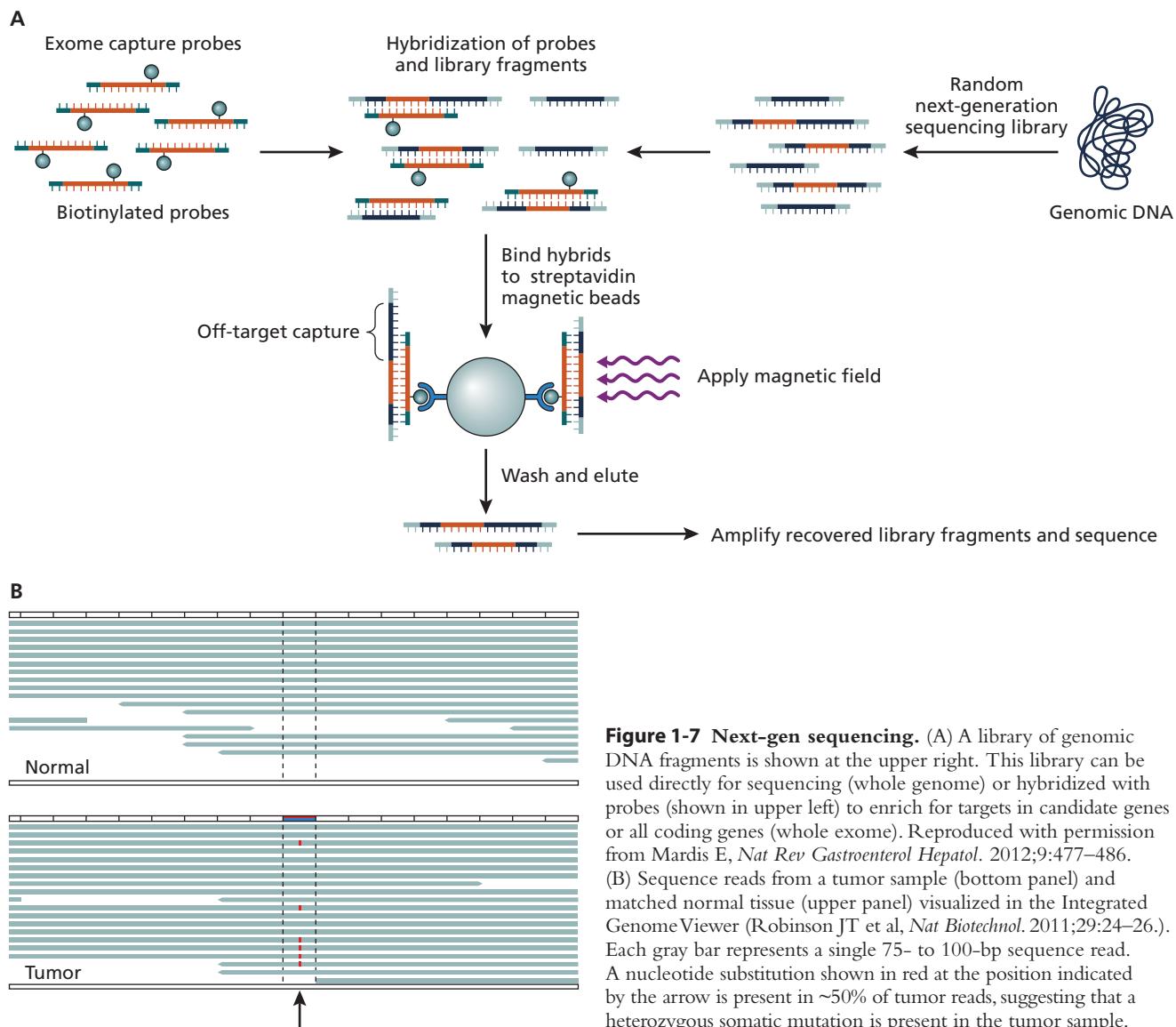


Figure 1-7 Next-gen sequencing. (A) A library of genomic DNA fragments is shown at the upper right. This library can be used directly for sequencing (whole genome) or hybridized with probes (shown in upper left) to enrich for targets in candidate genes or all coding genes (whole exome). Reproduced with permission from Mardis E, *Nat Rev Gastroenterol Hepatol*. 2012;9:477–486. (B) Sequence reads from a tumor sample (bottom panel) and matched normal tissue (upper panel) visualized in the Integrated Genome Viewer (Robinson JT et al, *Nat Biotechnol*. 2011;29:24–26.). Each gray bar represents a single 75- to 100-bp sequence read. A nucleotide substitution shown in red at the position indicated by the arrow is present in ~50% of tumor reads, suggesting that a heterozygous somatic mutation is present in the tumor sample.

variants that are often generated by sequencing the entire exome or genome. Also, some protein-coding genes are not yet annotated and therefore will be excluded from commercially available reagents used to capture the target DNA. These assays can be restricted further to panels of genes by first amplifying the genes of interest (by PCR) or by hybridizing the DNA to oligonucleotide probes covering the region of interest, followed by next-gen sequencing. This is an efficient and cost-effective approach to interrogate a number of targets in parallel from a single sample (eg, genes that are recurrently mutated in hematologic malignancies). Further modifications of the workflow allow for detection of chromatin marks across the genome (eg, transcription factor binding sites, histone modifications) by using antibodies to immunoprecipitate the region of interest, followed

by next-gen sequencing (**ChIP-Seq**). Next-generation sequencing-based assays can also be used to identify various epigenetic marks. For example, bisulfite sequencing is used to quantify the extent of cytosine methylation across the genome. In this method, sodium bisulfite exposure converts unmethylated cytosines to uracil, leaving methylated cytosines unaffected. RNA templates can be used to generate DNA libraries for sequencing (**RNA-seq**), allowing for the quantification of RNA abundance and for the detection of **chimeric** RNAs or alternatively spliced products. Finally, multiple approaches to determine chromatin accessibility and long-range protein–protein interactions have been developed using next-gen sequencing. Assay for transposase-accessible chromatin using sequencing (**ATAC-seq**) utilizes a mutated hyperactive transposase, an

enzyme that catalyzes the movement of transposon DNA elements to other parts in the genome. The high activity of the mutant enzyme allows for highly efficient cutting of exposed DNA and simultaneous ligation of adapters. Adapter-ligated DNA fragments are then isolated, amplified by PCR and used for next-gen sequencing. By isolating exposed DNA, the technique identifies areas of the genome where the chromatin is accessible, an indicator of genomic regions free of nucleosomes. Thus, enrichment of sequences indicates absence of DNA-binding proteins or nucleosomes in the region. These regions can be further categorized into regulatory elements—such as promoters, enhancers, and insulators—by integrating further genomic and epigenomic data, including information about histone modifications or evidence for active transcription. Chromosome conformation capture techniques are a set of molecular biology methods used to analyze the spatial organization of chromatin in a cell. These methods quantify the number of interactions between genomic loci that are nearby in three-dimensional space but may be separated by long distances in the linear genome. Such interactions may result from biological functions, such as promoter-enhancer interactions. **Hi-C** is one of these techniques that quantifies interactions between all possible pairs of fragments simultaneously. Briefly, cell genomes are cross-linked with a fixative to “freeze” interactions between genomic loci. The genome is then cut into fragments using restriction enzymes and a ligase is added to cross-link interacting fragments. Interacting loci fragments then undergo library preparation followed by high throughput sequencing.

Although the cost of sequence production has fallen dramatically in recent years, the storage, analysis, and interpretation of these large data sets still pose significant challenges.

Methods to study protein abundance

Proteins are the effectors of most cellular functions. Genetic defects perturb normal cellular functions because they result in changes in the level or function of the proteins they encode. Characterizing proteins expressed on cell surfaces and within cells is critical for identifying hematopoietic and immune cell subsets and is a cornerstone of diagnosing a wide variety of hematologic malignancies. Evaluation of protein expression abundance for diagnostic and therapeutic purposes is routinely performed using **flow cytometry** of suspension cells as well as **immunohistochemistry (IHC)** analysis of tissue sections. In addition, an **enzyme-linked immunosorbent assay (ELISA)** can be utilized to detect proteins such as peptides, proteins, antibodies and hormones in liquid or

tissue material. Many proteins undergo extensive post-translational modifications that influence their activity and function, including cleavage, chemical modification such as phosphorylation and glycosylation, and interaction with other proteins. These posttranslational events are not encoded by the genome and are not revealed by genomic analysis or gene expression profiling. **Proteomics** is the systematic study of the entire complement of proteins derived from a cell population.

IHC, flow cytometry, and ELISA analyses are heavily utilized in clinical diagnostic hematopathology. IHC analysis is the process of visualizing the expression of a protein within a section of tissue through the use of antibodies selective to a specific antigen. Most commonly, the antibody utilized in IHC is conjugated to an enzyme, such as peroxidase, which catalyzes a color-producing reaction allowing visualization of the antibody–antigen interaction. Because IHC is performed on a tissue section, the architecture of the tissue and cellular relationships in tissue is well preserved. In addition, IHC is performed on tissue that has been preserved through the process of fixation, most commonly using paraformaldehyde, allowing IHC analysis on archival tissue. IHC immunophenotyping is routinely used to differentiate subtypes of acute leukemias and lymphomas using panels of defined antibodies.

Similar to IHC, in ELISA, proteins are incubated with an antibody linked to an enzyme where the abundance of the protein is indicated by the extent of the enzymatic activity. Unlike IHC, however, ELISA is performed on any source where protein can be extracted and is routinely used to detect peptides, proteins, antibodies and hormones in clinical materials.

In contrast to IHC and ELISA, flow cytometry is utilized to characterize protein expression on cells obtained viably in single cell suspension. In flow cytometry, antibodies conjugated to a fluorescent protein bind to a cell surface (and/or in a cell that has been permeabilized for detection of proteins within a cell) and are passed through an electronic detection apparatus to enumerate the number of cells expressing that protein. In this manner, panels of antibodies with different fluorescent proteins are utilized to characterize numbers of proteins simultaneously on peripheral blood, bone marrow aspirate, and tissue fluid samples. In addition, flow cytometry provides quantitative information and has a level of sensitivity that allows it to be used for testing of minimal residue disease following treatment (described below). For both flow cytometry and IHC, variations of these techniques have been developed recently using antibodies conjugated to metal ions which allows for the use of much larger number of antibodies simultaneously.

Proteomic analysis relies on complex bioinformatic tools applied to mass spectroscopy data. In general, these techniques require some sort of separation of peptides, usually by liquid chromatography, followed by ionization of the sample and mass spectrometry. In matrix-associated laser desorption/ionization-time of flight mass spectrometry, the time of flight of the ions is detected and used to calculate a mass-to-charge ratio. The spectrum of mass-to-charge ratios present within a sample reflects the protein constituents within the sample. Supervised or unsupervised learning approaches, as described previously, are then used to identify patterns within the data. More recently, protein microarrays have been developed. Analytical protein microarrays are composed of a high density of affinity reagents (eg, antigens, antibodies) that can be used to detect the presence of specific proteins in a mixture. Functional protein microarrays contain a large number of immobilized proteins; these arrays can be used to examine protein-protein, protein-lipid, protein-nucleic acid, and enzyme-substrate interactions. Although all of these technologies hold enormous potential, clinical applications have yet to be realized.

Animal models

Analysis of both inherited and acquired diseases by reverse genetics has resulted in the identification of many disease-related genes for which the function is unknown. Once a disease-related gene has been identified, either by **linkage mapping** (eg, the gene for cystic fibrosis) or by identifying rearranged genes (eg, the *BCR* gene at the breakpoint of the Philadelphia chromosome), the challenge lies in identifying the function of the protein encoded by that gene and characterizing how changes in the gene can contribute to the disease phenotype. Understanding the role of these genes and their encoded proteins has been aided greatly by the development of techniques to alter or introduce these genes in mice using recombinant DNA technology.

Mice can be produced that express an exogenous gene and thereby provide an *in vivo* model of the gene's function. Linearized DNA is injected into a fertilized mouse oocyte pronucleus, and the oocyte is then reimplanted into a pseudopregnant mouse. The resultant **transgenic mice** then can be analyzed for phenotypes induced by the exogenous gene. Placing the gene under the control of a strong **constitutive promoter**, which is active in all tissues, allows for the assessment of the effect of widespread overexpression of the gene. Alternatively, placing the gene under a tissue-specific promoter will elucidate the function of that gene in an isolated tissue. A third approach is to use the control elements of the gene to drive the expression of a gene that can be detected by chemical, immunologic,

or functional means. For example, the promoter region of a gene can be joined to the green fluorescent protein cDNA, and expression of this reporter can be assessed in various tissues in the resultant transgenic mouse. Use of such a reporter gene will show the normal distribution and timing of the expression of the gene from which the promoter elements are derived. These transgenic mice contain multiple copies of exogenous genes that have inserted randomly into the genome of the recipient and thus may not mimic physiologic levels or spatiotemporal expression of the gene. In contrast, the endogenous genetic locus of a gene can be manipulated in totipotent embryonic stem (ES) cells by targeted recombination between the locus and a plasmid carrying an altered version of that gene that changes or disrupts its function. If a plasmid contains that altered gene with enough flanking DNA identical to that of the normal gene locus, **homologous recombination** will occur at a low rate; however, cells undergoing the desired recombination can be enriched by including a selection marker in the plasmid, such as the neomycin resistance gene. The correctly targeted ES cell is then introduced into the blastocyst of a developing embryo. The resultant animals will be chimeric, in that only some of the cells in the animal will contain the targeted gene. If the new gene becomes part of the germline, offspring can be bred to yield mice carrying the mutation in all cells. **Knockout mice** (homozygous for a null allele) can illuminate the function of the targeted gene by analyzing the phenotype of mice that lack the gene product. Similar approaches can be used to replace a normal mouse gene in ES cells with a version containing a point mutation, deletion, or other genetic variant to model abnormalities detected in patients with hematologic disorders.

Many genes of interest participate in pathways that are vital for viability or fertility; thus, constitutive knockout mice cannot be generated. Conditional gene modification using *Cre-loxP* technology allows the gene of interest to be altered in specific tissues or at specific times during development or postnatal life. This is accomplished by inserting the altered gene with flanking DNA containing *loxP* sites. If mice with paired *loxP* sites integrated into their genome are bred with a second strain of mice that express an enzyme called Cre recombinase, recombination will take place between the *loxP* sites, removing or rearranging the desired portion of the gene. Furthermore, expression of the Cre recombinase can be regulated in a tissue-specific manner by using an appropriate promoter or in a temporally restricted manner by using a promoter that is induced by treatment of the mice with a drug (such as tetracycline). The use of transgenic, knockout, and conditional knockout mice has been invaluable in elucidating

the function of large numbers of genes implicated in the pathogenesis of both inherited and acquired diseases.

Transgenic technology, however, is laborious, time consuming, and expensive. Some of these disadvantages can be circumvented by using rapidly reproducing and inexpensive organisms, such as zebrafish or yeast. Like transgenic mice, however, these models may not recapitulate human-specific pathophysiology. Newer technology using dedifferentiated somatic cells reprogrammed to become totipotent cells may overcome some of these obstacles. These cells, called **induced pluripotent stem (iPS) cells**, are produced by reprogramming adult somatic cells to become embryonic-like cells, which, in turn, can be further differentiated along specific lineages. The concrete demonstration that iPS cells may be used to treat disease was replacement of the sickle globin gene with a normal β -globin gene in mice. Corrected iPS cells from sickle mice were differentiated into hematopoietic progenitors in vitro, and these cells were transplanted into irradiated sickle mice recipients. Erythroid cells derived from these progenitors synthesized high levels of human hemoglobin A and corrected the sickle cell disease phenotype. Human iPS cells have been produced and hold great promise as research tools and possibly as a source of tissue replacement.

Given the time required for conventional gene targeting using homologous recombination, there has been great interest in the recent development of genome editing using **zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and CRISPR/Cas (clustered regulatory interspaced short palindromic repeat/Cas-based RNA-guided DNA endonucleases)**. Each of these techniques makes use of a nuclease that induces DNA breaks and then stimulates DNA repair in a way that allows for creation of specific mutations and/or inclusions of novel sequences of DNA. The nucleases are linked to sequence-specific DNA binding modules that allow for creation of mutations in specific locations of the genome. These techniques have allowed for rapid generation of knockout and knockin mice in embryonic or somatic stem cells to rapidly create genetically engineered animal models.

Clinical applications of DNA technology in hematology

Molecular biology has revolutionized the understanding of molecular pathogenesis of disease in ways that have profoundly affected the diagnostic armamentarium of the hematologist. Several examples of how molecular studies are used for diagnosis and clinical decision-making in hematology are described in this section.

Applications to germline (inherited) mutations

Hemoglobinopathies and thalassemias

One of the best examples of the use of molecular techniques in benign hematology is in the diagnosis of hemoglobinopathies and thalassemia. Although the most common hemoglobin variants (ie, Hb S, Hb C, Hb D) typically are diagnosed using nonmolecular methods, such as high-performance liquid chromatography or protein electrophoresis, molecular testing can be useful in several settings, including the characterization of uncommon variants, family screening studies, and prenatal diagnosis. Hemoglobin variants may be detected by a variety of techniques, including PCR using allele-specific primers designed to detect specific mutations or sequencing studies of the *HBA1/A2* and *HBB* loci. Molecular techniques are particularly valuable in the diagnosis of α -thalassemia, which usually is caused by one of several variably sized deletions that result in the loss of one or both *HBA* genes in the α -globin locus. In the neonatal period, α -thalassemia may be recognized by the presence of Hb Bart's (4 tetramers) on electrophoresis or high-performance liquid chromatography, but laboratory diagnosis after the neonatal period requires molecular techniques. Deletions of the α -globin locus can be detected by gap-PCR, which uses PCR primers that bind to either side of a deletion breakpoint. In the absence of the corresponding deletion, the primers are too far apart to yield an amplifiable product. When a deletion is present, however, an abnormal amplicon is detected.

Pharmacogenomics

Pharmacogenomics is the study of how inherited genetic variation affects the body's response to drugs. The term comes from the words *pharmacology* and *genomics* and is thus the intersection of both disciplines. For instance, homozygous germline polymorphisms in the thiopurine methyltransferase (TPMT) gene result in loss of functional protein and predispose ALL patients to severe hematologic toxicity unless the dose of mercaptopurine is reduced by 90% to 95% of normal. Heterozygote individuals also require dose reductions to a lesser extent than homozygotes. PCR-based studies may be performed to identify the presence of alleles associated with decreased TPMT function.

Applications to somatic (acquired) molecular abnormalities

The power of molecular biology to provide important insights into the basic biology of disease is perhaps most dramatically shown by the evolving concepts of malignancy. Several examples of how molecular techniques have enhanced our understanding of the pathogenesis of

hematologic malignancies, as well as their diagnosis and treatment, are provided in the following sections.

Gene rearrangement studies in lymphoproliferative disease: T-cell and B-cell rearrangements

During the development of a mature lymphoid cell from an undifferentiated stem cell, somatic rearrangements of the immunoglobulin and T-cell receptor loci take place, resulting in an extensive repertoire of composite genes that creates immense immunoglobulin and T-cell diversity. These somatic rearrangements result in deletion of intervening DNA sequences between gene segments in the immunoglobulin and T-cell receptor loci. The details of this process in lymphocyte ontogeny are further outlined in Chapter 21.

Rearrangements in immunoglobulin and T-cell receptor genes can be detected by either Southern blotting or PCR; however, PCR-based approaches using standardized, comprehensive primer sets such as those developed by the EuroClonality consortium (so called BIOMED-2 primers) are now preferentially used in the clinical setting due to the fact that they are more rapid, require less DNA, and can be performed on archived formalin-fixed, paraffin-embedded (FFPE) tissue. PCR-based techniques targeting *IGH* and *IGK* loci for B-cell rearrangements and *TCRG* and *TCRB* for T-cell rearrangements are used to confirm the presence of clonal lymphocyte populations in the peripheral blood, such as in T-cell large granular lymphocyte disorders, and also are powerful ancillary techniques for hematopathologists in the diagnosis of lymphoproliferative disorders from FFPE tissue (Figure 1–8). Despite their power, molecular clonality studies should be carefully interpreted in the context of the clinical, morphologic, and immunophenotypic diagnosis. Clonal proliferations may occur in some reactive conditions as well as in malignant neoplasms. For example, clonal T-cell populations may be detected in the setting of viral infections, such as with Epstein-Barr virus or cytomegalovirus, and clonal B-cell populations may be detected in some benign lymphoid proliferations, such as marked follicular hyperplasia. Furthermore, false-positive PCR results may occur in several circumstances; for example, when very small tissue samples are used, a few reactive T cells in the sample might result in the appearance of oligoclonal bands. False-negative results may occur as a result of tissue sampling, poor PCR amplification, or lack of detection of specific rearrangements using standardized primer sets.

Identification of cryptic translocations in pediatric leukemia: prognostic significance

Several fusion events in pediatric acute leukemia that carry prognostic significance are not detected by standard

cytogenetic techniques and require molecular testing for identification. Examples include the *ETV6-RUNX1* fusion that is present in ~20% of children with pre–B-cell ALL and confers a favorable prognosis, as well as high-risk fusion events found in AML—such as *MLL-AF10*, *CBFA2T3-GLIS2*, *NUP98-KDM5A*, and *NUP98-NSD1*, among others. These can be detected by FISH, RT-PCR, or next-gen sequencing approaches such as RNA-seq or whole genome sequencing. The identification of these lesions is important because they impact the intensity of chemotherapy treatment given to the patient up front, as well as the recommendation for stem cell transplant in first remission.

Prognostically significant mutations in normal karyotype acute myeloid leukemia

Up to 40% of AML cases have no chromosomal abnormalities visible by conventional karyotyping. The prognosis in these cases can be further refined by molecular testing for mutations in various recurrently mutated genes, including *NPM1*, *FLT3*, *CEBPA*, *DNMT3A*, and others. *NPM1* mutations, usually a 4-bp insertion in exon 12, are found in approximately 35% of cases of AML. *FLT3* mutations include variably sized duplications (internal tandem duplications) or point mutations in the kinase domain and are found in approximately one-third of AML cases. Mutations in *CEBPA* are diverse and can be found in approximately 10% of AML cases. Cases of AML with a normal karyotype and a mutant *NPM1*/wild-type *FLT3* genotype or harboring biallelic *CEBPA* mutations are associated with a favorable prognosis. Furthermore, studies have suggested that AML with mutated *NPM1* or mutated *CEBPA* each represent distinct clinicopathologic entities. Using multivariate analysis, mutations in *DNMT3A* have emerged as powerful predictors of poor prognosis in AML. Currently, a limited number of genes are routinely tested in AML patients by conventional (Sanger) sequencing or PCR assays. The use of next-gen sequencing panels has allowed for improved prognostic assessment and treatment selection based on testing a larger number of recurring mutations in myeloid neoplasms, including AML, MDSs, and myeloproliferative neoplasms.

Minimal residual disease monitoring

The development of PCR has markedly increased the sensitivity of tests available for the monitoring of MRD in myeloid and lymphoid neoplasms. With the availability of real-time PCR, the relative abundance of specific transcripts can now be monitored to assess trends of increase or decrease over time. For example, real-time quantitative RT-PCR is used routinely in CML to risk-stratify patients

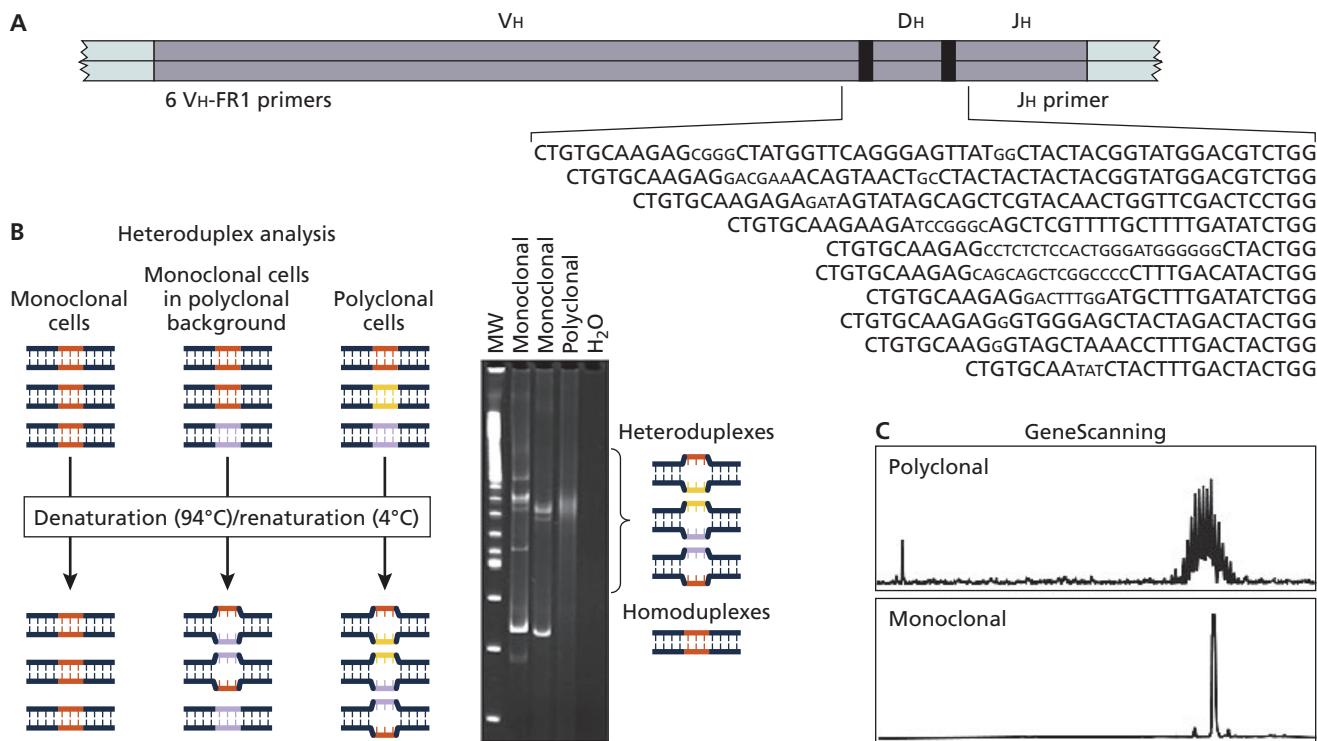


Figure 1-8 Schematic diagram of heteroduplex analysis and GeneScanning of PCR products, obtained from rearranged Ig and TCR genes. (A) Rearranged Ig and TCR genes (*IGH* in the example) show heterogeneous junctional regions with respect to size and nucleotide composition. Germline nucleotides of V, D, and J gene segments are given in large capitals and randomly inserted nucleotides in small capitals. The junctional region heterogeneity is employed in heteroduplex analysis (size and composition) and GeneScanning (size only) to discriminate between products derived from monoclonal and polyclonal lymphoid cell populations. (B) In heteroduplex analysis, PCR products are heat denatured (5 min, 94°C) and subsequently rapidly cooled (1 h, 4°C) to induce duplex (homo- or heteroduplex) formation. In cell samples consisting of clonal lymphoid cells, the PCR products of rearranged *IGH* genes give rise to homoduplexes after denaturation and renaturation, whereas in samples that contain polyclonal lymphoid cell populations the single-strand PCR fragments will mainly form heteroduplexes, which result in a background smear of slowly migrating fragments upon electrophoresis. (C) In GeneScanning, fluorochrome-labeled PCR products of rearranged *IGH* genes are denatured prior to high-resolution fragment analysis of the resulting single-stranded fragments. Monoclonal cell samples give rise to PCR products of identical size (single peak), whereas in polyclonal samples many different *IGH* PCR products are formed, which show a characteristic Gaussian size distribution. Reprinted by permission from Macmillan Publishers Ltd (van Dongen J, et al. *Leukemia*. 2003;17:2257–2317).

based on transcript quantity rather than simply the presence or absence of a transcript (as discussed previously in this chapter). The accuracy and reliability of real-time quantitative PCR as a measure of *BCR-ABL1* transcript level depends on the quality control procedures carried out by the laboratory. Normalization of the results to an appropriate control gene is required to compensate for variations in RNA quality and the efficiency of the **reverse transcriptase** reaction. *BCR* and *ABL1* have been used as control genes, and both seem to be suitable because they are expressed at low levels and have similar stability to *BCR-ABL1*. The introduction of internationally recognized reference standards now has allowed for reporting of results on the International Scale, which allows for direct comparisons of results among laboratories, even those using different control genes. A major molecular response to ima-

tinib has been defined as a 3-log reduction in *BCR-ABL1* transcripts (*BCR-ABL1*/reference gene) compared with a standardized baseline obtained from patients with untreated newly diagnosed CML, corresponding to 0.1% on the International Scale.

In similar fashion, PCR analysis of immunoglobulin or T-cell receptor gene rearrangements allow the detection of residual disease in the blood or bone marrow of patients who have undergone treatment of a lymphoid malignancy. Because each gene rearrangement is unique, however, the PCR detection of gene rearrangements at this level of sensitivity is labor intensive. PCR of tumor tissue is performed using primers based on consensus sequences shared by the variable and joining regions of the appropriate locus (immunoglobulin or T-cell receptor genes). The specific rearrangement must then be sequenced so that an oligonucleotide

specific to the unique rearrangement in that patient's tumor can be synthesized. PCR can then be performed using this **allele-specific oligonucleotide**, with adequate sensitivity to detect 1 in 10^6 cells. As these assays become increasingly available, they will play an important role in estimating prognosis and determining eligibility for autologous transplantation and other therapeutic modalities.

In addition to MRD monitoring by molecular monitoring of DNA/cDNA retrieved from hematopoietic cells, flow cytometric analysis to detect residual leukemic cells in peripheral blood or bone marrow has also been heavily used for MRD monitoring in ALL and more recently in pediatric AML (amongst other hematological malignancies). Studies in pediatric T-lineage ALL demonstrate that while molecular techniques are more sensitive than flow cytometry in detecting residual disease, this fails to predict relapse more accurately. Similarly, in pediatric AML, MRD levels of 0.01% by flow cytometry do not identify patients at lower risk for relapse compared to those that achieve less than 0.1% following induction chemotherapy, suggesting that a threshold that predicts relapse exists beyond which higher levels of sensitivity do not provide prognostic relevance. Further efforts to understand the sensitivity of flow cytometric MRD detection relative to molecular techniques are underway. As the two techniques are complementary, and there exist cases that are not suitable for one or the other, both approaches can be used in tandem to provide optimal prognostic information.

Expression profiling: applications to diagnosis and treatment

Gene expression microarray studies have facilitated the classification of lymphomas and outcome prediction for specific patient populations with this disease. For example, expression profiling was used to create a prediction model that identified two categories of patients with diffuse large B-cell lymphoma: germinal center and activated B-cell types, which carry favorable and unfavorable prognoses, respectively. In addition, expression profiling studies have identified genes overexpressed in patients with poor prognosis, some of which may represent potential therapeutic targets.

Applications to stem cell transplantation

Human leukocyte antigen typing for stem cell transplantation

Molecular techniques have been important to the further understanding of the diversity of human leukocyte antigen (HLA) genotypes. Serologic testing for HLA antigens often identifies broad groups of cross-reactive antigens. Because there is an increased incidence of severe graft-versus-host disease in patients who receive transplants from sero-

logically compatible but genotypically incompatible unrelated donors, it is important to identify the individual antigens within these cross-reactive groups. Genotypic HLA typing can be achieved by PCR amplification of the HLA locus, followed by hybridization to specific oligonucleotides corresponding to the different alleles within a given cross-reactive group. Such genotyping is much more predictive of successful transplantation and the risk of graft-versus-host disease than serologic study or the mixed lymphocyte assay, and it has supplanted these assays for the identification of optimal donors, especially unrelated donors. Comprehensive genotyping using SNP arrays may improve HLA matching. This is discussed in detail in Chapter 12.

Analysis of bone marrow engraftment

When donor and recipient are of opposite sex, the assessment of donor engraftment is based on conventional cytogenetics and is relatively straightforward. When donor and recipient are of the same sex, RFLP analysis of donor and recipient bone marrow allows the detection of polymorphic markers to distinguish DNA from the donor and recipient. After transplantation, RFLP analysis of recipient peripheral blood cells then can be used to document engraftment, chimerism, graft failure, and disease relapse. In most centers, PCR amplification and genotyping of short tandem repeat or variable number tandem repeat sequences that are polymorphic between donor and recipient pairs are now used to assess chimerism.

Applications to novel therapies

Antisense and RNA interference therapy

The recognition that abnormal expression of oncogene products plays a role in malignancy has led to the proposal that suppression of that expression might reverse the neoplastic phenotype. One way of blocking mRNA expression is through the use of **antisense oligonucleotides**. These are short pieces of single-stranded DNA or RNA, 17 to 20 bases long, which are synthesized with a sequence complementary to the transcription or the translation initiation site in the mRNA. These short single-stranded species enter the cell freely, where they complex to the mRNA through the complementary sequence. Investigation of the mechanism of action of antisense oligonucleotides led to the discovery that naturally occurring double-stranded RNA molecules suppress gene expression better than antisense sequences and helped to unravel the mechanism of RNA interference. RNA interference has significant advantages over antisense therapy in that much lower concentrations are required. Numerous studies are under way in hematologic diseases; however, methods for delivery of siRNAs are still far from perfect. In one study, adult stem

cells from sickle cell patients were infected with a viral vector carrying a therapeutic γ -globin gene harboring an embedded siRNA precursor specific for sickle β -globin. The newly formed red blood cells made normal hemoglobin and suppressed production of sickle β -globin. In another study, a retroviral system for stable expression of siRNA directed to the unique fusion junction sequence of *ETV6-PDGFRB* resulted in profound inhibition of *ETV6-PDGFRB* expression and inhibited proliferation of *ETV6-PDGFRB*-transformed cells. When applied to mice, this strategy slowed tumor development and death in mice injected with these cells compared with cells not containing the siRNA. Stable siRNA expression sensitized transformed cells to the *PDGFRB* inhibitor imatinib, suggesting that stable expression of siRNAs, which target oncogenic fusion genes, may potentiate the effects of conventional therapy for hematologic malignancies.

Gene therapy

The application of gene therapy to genetic hematologic disorders has long been an attractive concept. In most cases, this involves insertion of normal genes into autologous hematopoietic stem cells with subsequent transplantation back into the patient. Candidate hematologic diseases for such therapy include hemophilia, sickle cell disease, thalassemia, and severe combined immune deficiency syndrome. Rapid advances in technology for the separation of hematopoietic stem cells and techniques of gene transfer into those cells have advanced efforts toward this goal, and many clinical trials have been completed. Although significant methodologic hurdles remain, research in this field continues to move forward. It should be recognized, however, that correction of such diseases as hemophilia, sickle cell disease, and thalassemia requires efficient gene transfer to a large number of hematopoietic stem cells with high levels of expression of the β -globin gene in erythroid precursors. Long-term repopulating stem cells have been relatively resistant to genetic modification; thus, many investigators have focused on gene therapy applications in which low levels of expression could restore patients to health. A major impediment to successful gene therapy has been the lack of gene delivery systems that provide safe, efficient, and durable gene insertion and that can specifically target the cells of interest. An important safety concern with viral vectors that integrate into the host genome is the potential to activate oncogenes or inactivate tumor suppressor genes by insertional mutagenesis. Currently used approaches include retroviral vectors, adenoviral vectors, other viral vectors, and nonviral vectors. One of the more recent successes in the field that has overcome these challenges has been recently reported for

hemophilia B. High expression levels of a functional factor IX was found in patients treated with a single injection of adeno-associated viral vector containing a hyperfunctional factor IX variant gene. All participants in the study had sustained factor IX levels one-third of the normal value, with dramatically reduced annual bleeding rates.

Glossary

alleles Alternative forms of a particular gene.

allele-specific oligonucleotide An oligonucleotide whose sequence matches that of a specific polymorphic allele. For example, oligonucleotides matching the sequence of unique immunoglobulin or T-cell receptor gene rearrangements that are used for polymerase chain reaction (PCR) detection of minimal residual disease (MRD).

alternative splicing Selective inclusion or exclusion of certain exons in mature RNA by utilization of a varied combination of splicing signals.

antisense oligonucleotides Oligonucleotides with a base sequence complementary to a stretch of DNA or RNA coding sequence.

ATAC seq High-throughput sequencing approach to measure DNA accessibility.

capping Addition of the nucleotide 7-methylguanosine to the 5' end of mRNA. This is a structure that appears to stabilize the mRNA.

chimera An organism containing two or more different populations of genetically distinct cells (as in chimeric mice generated by microinjection of embryonic stem cells into a developing blastocyst or chimerism of donor and recipient cells after allogeneic stem cell transplantation). Also used to describe transcripts that fuse coding sequences from different genes as a result of chromosomal rearrangements.

chimeric antigen receptor T cells (CAR T cells) Genetically modified T cells engineered to express an artificial T-cell receptor that recognizes a specific tumor-associated antigen.

ChIP-Seq A combination of chromatin immunoprecipitation followed by next-gen sequencing used to identify protein-DNA interactions.

chromatin A complex of genomic DNA with histone and non-histone proteins.

chromosome A large linear DNA structure tightly complexed to nuclear proteins.

cis-acting regulatory elements Sequences within a gene locus, but not within coding sequences, that are involved in regulating the expression of the gene by interaction with nuclear proteins.

clonal Arising from the expansion of a single cell.

coding sequence The portion of the gene contained within exons that encodes the amino acid sequence of the protein product.

codon The 3-nucleotide code that denotes a specific amino acid.

comparative genomic hybridization (CGH) A technique allowing for the detection of subtle chromosomal changes (deletions, amplifications, or inversions that are too small to be detected by conventional cytogenetics techniques).

complementary Sequence of the second strand of DNA that is determined by strict purine–pyrimidine base pairing (A–T; G–C).

complementary DNA (cDNA) Double-stranded DNA product from an RNA species. The first strand is synthesized by reverse transcriptase to make a DNA strand complementary to the mRNA. The second strand is synthesized by DNA polymerase to complement the first strand.

constitutive promoter A promoter that drives high-level expression in all tissues.

copy number variant A segment of DNA at least 1 kb in length that varies in copy number between individuals.

CRISPR/Cas (clustered regulatory interspaced short palindromic repeat/Cas-based RNA-guided DNA endonucleases) A technology which combines the Cas DNA nuclease with the sequence-specific DNA recognition module of CRISPR to create targeted genetic alterations in DNA.

cytogenetics The study of the chromosomal makeup of a cell.

degenerate Characteristic of the genetic code whereby more than one codon can encode the same amino acid.

Dicer A component of the processing mechanism that generates microRNAs and siRNAs.

Drosha A component of the processing mechanism for formation of microRNAs.

enhancer A *cis*-acting regulatory sequence within a gene locus that interacts with nuclear protein in such a way as to increase the expression of the gene.

enzyme-linked immunosorbent assay (ELISA) A method used to detect and quantify proteins (such as peptides, proteins, antibodies and hormones) using an antibody linked to an enzyme.

epigenetics Changes in gene expression caused by mechanisms other than alteration of the underlying DNA sequence. Includes DNA methylation and histone modification. The changes are heritable in daughter cells but can be modified pharmacologically (eg, methyltransferase inhibitors, histone deacetylase inhibitors) or by normal enzymatic processes.

exome The set of all protein-coding portions of genes (exons) in the genome.

exon The portion of a structural gene that encodes protein.

flanking sequences DNA sequences lying 5' and 3' of a structural gene that frequently contain important regulatory elements.

flow cytometry A method to quantify protein expression on cells using antibody where the cell with antibody bound are

passed in a single suspension through a machine with a laser to detect abundance.

fluorescence in situ hybridization (FISH) High-resolution mapping of genes by hybridization of chromosome spreads to biotin-labeled DNA probes and detection by fluorescent-tagged avidin.

frameshift mutation A mutation within the coding sequence of a gene that results from deletion or insertion of a nucleotide that disrupts the 3-base codon structure of the gene, thereby altering the predicted amino acid sequence of the protein encoded by that gene.

gene A functional genetic unit responsible for the production of a given protein, including the elements that control the timing and the level of its expression.

gene expression profile Analysis of the global expression of a collection of cells using hybridization of mRNA to microarrays.

gene regulation A process controlling the timing and level of expression of a gene.

genetic code The system by which DNA encodes specific proteins through 3-nucleotide codons, each encoding a specific amino acid.

genomics The study of the entire DNA sequence of organisms and interactions among various genetic loci.

Hi-C A next-generation sequencing approach to identify long-range chromatin interactions genome wide.

homologous recombination Alteration of genetic material by alignment of closely related sequences. In targeting genes by homologous recombination, plasmids that contain altered genes flanked by long stretches of DNA that match the endogenous gene are introduced into embryonic stem cells. A rare recombination event will cause the endogenous gene to be replaced by the mutated gene in the targeting plasmid. This is the means by which knockout mice are obtained.

immune checkpoint inhibitors Monoclonal antibodies directed against molecules that mediate T-cell inhibitory signals such as CTLA-4, PD-1 and its ligand PD-L1.

immunohistochemistry (IHC) A method of detecting proteins in tissue sections using antibodies linked to substrates that allow for visual detection of antibody-protein binding abundance *in situ*.

imprinting A genetic process in which certain genes are expressed in a parent-of-origin-specific manner.

induced pluripotent stem (iPS) cells A type of pluripotent stem cell derived from a somatic cell that is generated by exposing the somatic cell to factors that reprogram it to a pluripotent state.

intron An intervening sequence of noncoding DNA that interrupts coding sequence contained in exons.

knockin mouse A mouse in which nucleotides have been inserted into the mouse genome to allow expression of protein not normally encoded by the mouse genome.

knockout mouse A mouse in which both of the copies of a gene have been disrupted by a targeted mutation. Such mutations are achieved by homologous recombination using plasmids containing the mutated gene flanked by long stretches of the normal endogenous gene sequence. Mice that are heterozygous in the germline for the targeted allele can be bred to generate mice that lack both copies of the normal (wild-type) gene.

leucine zipper Leucine-rich side chains shared by a group of transcription factors that allow protein–protein and protein–DNA interactions.

linkage mapping Analysis of a gene locus by study of inheritance pattern of markers of nearby (linked) loci.

methylation DNA modification by addition of methyl groups to cytosine residues within genomic DNA. Hypermethylation of clustered CpG groups in promoter regions (CpG islands) is a characteristic of transcriptionally inactive DNA; reduction in methylation is generally associated with increased transcriptional activity.

microarray A glass slide or silicon chip on which cDNAs or oligonucleotides have been spotted to allow for the simultaneous analysis of expression of hundreds to thousands of individual mRNAs. Hybridization of labeled cDNAs from a tissue of interest allows the generation of a gene expression profile.

microRNAs Small RNA molecules encoded in the genomes of plants and animals. These highly conserved, approximately 21-mer RNAs regulate the expression of genes both by changing stability of mRNAs as well as by translational interference.

missense mutation A mutation within the coding sequence of a gene that results from a single nucleotide change which alters the encoded amino acid leading to a change in protein function.

next-generation (next-gen) sequencing Massively parallel sequence production from single-molecule DNA templates.

noncoding sequences DNA sequences that do not directly encode protein.

nonsense mutation A nucleotide change converting an amino acid coding codon to a stop codon.

nonsense-mediated decay Nonsense mutation (premature stop codon) of one allele of an mRNA may result in degradation of the abnormal mRNA.

Northern blotting Analysis of RNA expression by gel electrophoresis, transfer to nitrocellulose or nylon filter, and hybridization to a single-stranded probe.

nucleic acid hybridization A technique of nucleic acid analysis via association of complementary single-stranded species.

nucleotide A basic building block of nucleic acids, composed of a sugar moiety linked to a phosphate group and a purine or pyrimidine base.

oligonucleotide A short single-stranded DNA species, usually composed of 15 to 20 nucleotides.

oncogene Cellular gene involved with normal cellular growth and development, the altered expression of which has been implicated in the pathogenesis of the malignant phenotype.

partial uniparental disomy A situation in which two copies of a chromosome, or part of a chromosome, are derived from one parent and no copies derive from the other parent. In a somatic cell, this can result in progeny with two copies of the wild-type allele or two copies of the mutant allele.

polyadenylation Alteration of the 3' end of mRNA by the addition of a string of adenosine nucleotides ("poly-A tail") that appear to protect the mRNA from premature degradation.

polymorphism A phenotypically silent mutation in DNA that is transmitted from parent to offspring.

pre-messenger RNA Unprocessed primary RNA transcript from DNA, including all introns.

promoter Region in the 5' flanking region of a gene that is necessary for its expression; includes the binding site for RNA polymerase II.

proteomics The systematic study of the entire complement of proteins derived from a cell population.

purine Either of two of the bases found in DNA and RNA: adenine and guanine.

pyrimidine One of the following bases found in DNA and RNA: cytosine and thymine in DNA; cytosine and uracil in RNA.

quantitative PCR PCR in which the product is quantitated in comparison to the PCR product resulting from a known quantity of template. This allows quantitation of the template in the reaction; it can, for example, allow an estimate of the degree of contamination with tumor cells in a cell population.

real-time PCR An automated technique for performing quantitative PCR using a fluorogenic reporter to detect levels of target sequences during early cycles of the PCR reaction.

restriction endonucleases Enzymes produced by bacteria that cleave double-stranded DNA at specific recognition sequences.

restriction fragment-length polymorphism (RFLP) A polymorphism in which a silent mutation occurs within the recognition sequence for a restriction endonuclease. This results in an alteration in the size of the DNA fragment resulting from digestion of DNA from that DNA locus.

reverse transcriptase An enzyme encoded by retroviruses that mediates conversion of RNA to complementary DNA.

reverse transcriptase polymerase chain reaction (RT-PCR) Amplification of RNA sequences by conversion to cDNA by reverse transcriptase, followed by the polymerase chain reaction.

ribosome A ribonuclear protein complex that binds to mRNA and mediates its translation into protein by reading the genetic code.

RNA expression array An array-based technique used to determine the abundance of each of the known mRNAs (the gene expression profile) in a group of cells.

RNA-induced silencing complex (RISC) A multiprotein complex that combines with microRNAs to target complementary mRNA for degradation or translation inhibition.

RNA polymerase II An enzyme that mediates transcription of most structural genes.

RNA-seq Next-gen sequencing using RNA templates.

silencer A *cis*-acting regulatory sequence within a gene locus that interacts with nuclear protein in such a way as to decrease the expression of the gene.

single-nucleotide polymorphism (SNP) Naturally occurring inherited genetic variation between individuals at the level of single nucleotides.

small interfering RNAs (siRNAs) Small RNAs that act in concert with large multiprotein RISCs to cause cleavage of complementary mRNA or prevent its translation.

Southern blotting Analysis of DNA by gel electrophoresis, transfer to nitrocellulose or nylon filter, and hybridization to single-stranded probe.

splicing The process by which intron sequences are removed from pre-mRNAs.

telomeres Nucleoprotein structures at the ends of chromosomes that protect chromosome ends from degradation and fusion.

termination codon One of three codons that signal the termination of translation.

trans-acting factor A protein that interacts with *cis*-acting regulatory region within a gene locus to regulate transcription of that gene. Also called transcription factor.

transcription The process by which pre-mRNA is formed from the DNA template.

transcription activator-like effector nucleases (TALENs) Artificial restriction enzymes with sequence-specific DNA binding activity which can be utilized to create specific genetic alterations in DNA.

transcription factor A protein that interacts with *cis*-acting regulatory region within a gene locus to regulate transcription of that gene. Also called *trans*-acting factor.

transfer RNA (tRNA) Small RNA molecules that bind to the ribosome and covalently bind specific amino acids, allowing translation of the genetic code into protein.

transgenic mouse A mouse that expresses an exogenous gene (transgene) introduced randomly into its genome. Linearized DNA is injected into the pronucleus of a fertilized oocyte, and the zygote is reimplanted. Resultant mice will carry the transgene in all cells.

translation The process by which protein is synthesized from an mRNA template.

translocation breakpoint Site of junction of two aberrantly juxtaposed (translocated) chromosomal fragments.

tumor suppressor A gene that promotes tumor development when deleted or inactivated.

Western blotting Detection of specific proteins via binding of specific antibody to protein on a nitrocellulose or nylon membrane.

zinc finger A structural feature shared by a group of transcription factors. Zinc fingers are composed of a zinc atom associated with cysteine and histidine residues; the fingers appear to interact directly with DNA to affect transcription.

zinc finger nucleases Artificial restriction enzymes generated by fusing a zinc finger DNA binding domain to a DNA-cleavage domain for use in creating specific genomic alterations in DNA.

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Consultative hematology I: hospital-based and selected outpatient topics

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The online version of this chapter contains educational multimedia components on normal hematopoiesis and the mechanism of action of anticoagulants.

The role of the hematology consultant

A hematology consultant provides expert advice about the diagnosis and management of benign or malignant hematologic disorders to requesting physicians and other health care providers. A consultation request might involve an adult general medical patient, a child or adolescent, a pregnant woman, a perioperative patient, or an individual who is critically ill. Other consultative responsibilities of the hematologist may include serving on committees that maintain a formulary, developing clinical practice guidelines, establishing policies and procedures for transfusion services, or monitoring quality and efficiency. The setting of a consultation can be inpatient or outpatient and the timing emergent, urgent, subacute, or more planned. Imperative to an effective and efficient consultation, both the referring clinician and the hematology consultant must have a clear understanding of the extent of the clinical questions being asked, which in turn will guide the aim and comprehensiveness of the consult. In the era of rising health care costs, expert hematology consultation must seek to be cost effective by curtailing unwarranted diagnostic and therapeutic measures. With that in mind, the American Society of Hematology (ASH) Choosing

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Off-label drug use: Drs. Carpenter and Connell: corticosteroids and rituximab for use in TTP; ATIII and activated protein C for DIC; cyclophosphamide, corticosteroids, IVIG, and rituximab for CAPS; corticosteroids, intravenous immunoglobulin, rituximab, and thrombopoietin receptor agonists for pediatric ITP; rituximab for adult ITP and TTP; granulocyte-stimulating factor outside of severe congenital neutropenia. Desmopressin for use in platelet function disorders; desmopressin, recombinant FVIIa, prothrombin complex concentrate, activated prothrombin complex concentrate, fibrin glue, ε-aminocaproic acid, and tranexamic acid for use in surgical bleeding and reversal of direct oral anticoagulants; IVIG for posttransfusion purpura; IVIG for drug-induced ITP; conjugated estrogens for use in uremic bleeding; hemoglobin-based oxygen carriers for anemia in patients who refuse blood products; IVIG and erythropoietin for use in parvovirus-associated pure red cell aplasia; angiotensin-converting enzyme inhibitors and angiotensin receptor blockers for use in post–renal transplant erythrocytosis; rituximab for use in CD20⁺ posttransplant lymphoproliferative disorders.

Wisely campaign seeks to identify and educate clinicians on commonly performed tests or procedures within the realm of hematology that are unnecessary, not supported by evidence, duplicative, and potentially harmful (<http://www.hematology.org/Clinicians/Guidelines-Quality/502.aspx>). A clinical hematologist must understand the principles of effective consultation and the extreme importance of interphysician communication (Table 2-1). Consultants need to communicate effectively—not only with other staff physicians and consultants, but also with ancillary members of the health care team, house staff, fellows, students, and the patient and family. A commitment to effective communication ensures maximal compliance with recommendations and the highest quality of multidisciplinary patient care.

This chapter discusses some of the most common hematological consultations, including preoperative management of hematological disorders, inpatient and outpatient consultations, and specific issues pertaining to pediatric hematology.

Consultation for surgery and invasive procedures

CLINICAL CASE

A 34-year-old female with systemic lupus erythematosus (SLE) has been referred to your hematology clinic for perioperative management of her anticoagulation. She was recently diagnosed with antiphospholipid syndrome (APS) during the workup for a large right middle cerebral artery infarct 3 months ago. She is on warfarin with an international normalized ratio (INR) goal of 2.0 to 3.0 with approximately 80% time in therapeutic range. She now requires a tooth extraction for an abscessed tooth that has not responded to medical therapy. In light of the patient's APS and history of cerebrovascular accident, you judge her thrombotic risk to be high if warfarin is interrupted. Because of the risk of infection progressing, the surgery cannot be delayed. The oral surgeon is concerned about the patient's bleeding risk. You advise the patient to continue warfarin at the current dose and prescribe an adjuvant mouthwash containing ϵ -aminocaproic acid to control local bleeding.

Perioperative management of antithrombotic therapy

Hematologists are often consulted to provide recommendations on temporary interruption of antithrombotics for a surgery or procedure (see video file in online edition on mechanism of action of anticoagulants). The perioperative

management of patients taking antiplatelet or anticoagulant drugs is based on (i) an assessment of risk for perioperative bleeding and (ii) an assessment of the patient's risk for thromboembolism. These considerations are used to determine whether antithrombotic therapy should be interrupted prior to surgery and, if so, whether bridging anticoagulation should be considered.

Assessment of risk for perioperative bleeding

Bleeding risk is related to both surgical and host factors. Surgical factors include the location and extent of the intervention, the vascularity and fibrinolytic activity of the surgical bed, the compressibility of the site and the ability to achieve surgical hemostasis, and the possibility that the procedure may induce a hemostatic defect (eg, platelet dysfunction due to cardiopulmonary bypass). Host factors include the presence of an underlying congenital or acquired hemostatic defect and use of drugs that affect hemostasis.

A focused medical history should include a detailed personal history of abnormal bleeding; response to prior hemostatic challenges, such as surgeries, trauma, and childbirth; and comorbidities or use of medications that could affect hemostasis. Patients should be queried specifically about common procedures such as tooth extraction and tonsillectomy, which they may not think to mention unless prompted. Various bleeding assessment tools have been published with varying degrees of sensitivity and specificity for inherited bleeding disorders and may help guide who should undergo additional testing. A careful family history of bleeding is crucial, particularly in patients who may not have undergone extensive prior hemostatic challenges themselves. A targeted physical examination for stigmata of bleeding and evidence of comorbid conditions that may affect hemostasis, such as liver disease or a connective tissue or vascular disorder, should be performed as a complement to the history.

Preoperative hemostatic laboratory testing (ie, aPTT/PT) is neither cost effective nor informative in patients without a personal or family history suggestive of a bleeding disorder. However, if the history or physical examination is suggestive of a bleeding diathesis, preoperative testing should include a platelet count, prothrombin time (PT), and activated partial thromboplastin time (aPTT). Normal initial testing does not exclude a clinically important bleeding diathesis such as a platelet function defect, von Willebrand disease, mild factor deficiency, or a fibrinolytic disorder, and further testing should be guided by the clinical history and the results of the initial laboratory evaluation. Once a diagnosis has been established, a plan for perioperative hemostatic management should be developed

Table 2-1 Principles of effective consultation and interphysician communication

Principle	Comment
Determine the question that is being asked	The consultant must clearly understand the reason for the consultation
Establish the urgency of the consultation and respond in a timely manner	Urgent consultations must be seen as soon as possible (communicate any expected delays promptly); elective consultations should be seen within 24 hours
Gather primary data	Personally confirm the database; do not rely on secondhand information
Communicate as briefly as appropriate	Compliance is optimized when the consultant addresses specific questions with 5 succinct and relevant recommendations
Make specific recommendations	Identify major issues; limit the diagnostic recommendations to those most crucial; and provide specific drug doses, schedules, and treatment guidelines
Provide contingency plans	Briefly address alternative diagnoses; anticipate complications and questions
Understand the consultant's role	The attending physician has primary or ultimate responsibility; the consultant should not assume primary care or write orders without permission from the attending
Offer educational information	Provide relevant evidence-based literature or guidelines
Communicate recommendations directly to the requesting physician	Direct verbal contact (in person or by phone) optimizes compliance and minimizes confusion or error
Provide appropriate follow-up	Continue involvement and progress notes as indicated; officially sign off the case or provide outpatient follow-up

Adapted from Goldman L, Lee T, Rudd P. *Arch Intern Med.* 1983;143:1753–1755; Sears CL, Charlson ME. *Am J Med.* 1983;74:870–876; and Kitchens CS, Kessler CM, Konkle BA. Consultative Hemostasis and Thrombosis. 3rd ed. (Philadelphia, PA: Elsevier Saunders; 2013:3–15).

based on the nature and severity of the defect and the bleeding risk of the anticipated procedure. Although high-level evidence is lacking, a fibrinogen of at least 100 mg/dL and a platelet count of at least $50 \times 10^9/L$ is desired for moderate- to high-risk procedures. For neurosurgery and ophthalmologic procedures, it often is prudent to target a platelet count of at least $100 \times 10^9/L$.

A common preoperative hematology question is what to do with an isolated prolonged PTT in a patient without a bleeding history. The most common cause is the presence of a lupus anticoagulant. If the lupus anticoagulant testing is positive in this scenario, then no further workup is needed prior to proceeding with surgery because the bleeding history is the best predictor of ability to tolerate invasive procedures. In those without sufficient prior hemostatic challenges or negative lupus anticoagulant testing, further evaluation of the prolonged PTT must be completed prior to elective surgery in order to exclude the possibility of a clinically relevant factor deficiency.

Assessment of risk for thromboembolism

In general, patients may be classified as having a high, moderate, or low risk of perioperative thromboembolism. These categories correspond to an estimated annual thrombotic risk of >10%, 5% to 10%, and <5%, respectively. Individuals with mechanical mitral valves, atrial fibrillation and CHADS₂ scores of 5 or 6, recent (within

3 months) stroke or venous thromboembolism (VTE), or severe thrombophilia (eg, antithrombin deficiency or APLS) are considered high risk. Those with atrial fibrillation and CHADS₂ scores of 0 to 2, or a remote history of VTE more than 12 months before surgery and no other thrombotic risk factors, typically are classified as low risk. Individual patient factors not captured in this classification scheme, as well as type of surgery, should be considered in estimating an individual patient's perioperative thrombotic risk and whether bridging anticoagulation is necessary. This thrombotic risk must be weighed against the risk of surgical hemorrhage. For example, in patients with high risk of perioperative thrombosis, continuation of warfarin rather than bridging with heparin in those requiring pacemaker or implantable cardioverter-defibrillator surgery reduces clinically significant device-pocket hematomas without any difference in thromboembolic events. An assessment of hemorrhagic risk should take into account the propensity for bleeding associated with both the procedure and antithrombotic agent in question. The HAS-BLED score, which assigns 1 point each for hypertension, abnormal liver function, abnormal renal function, stroke, bleeding tendency, labile INRs while on warfarin, age >65, concomitant antiplatelet agent, or excess alcohol use, was evaluated in an observational registry study and scores ≥ 3 were most associated with bleeding even when warfarin was stopped and low-molecular-weight heparin

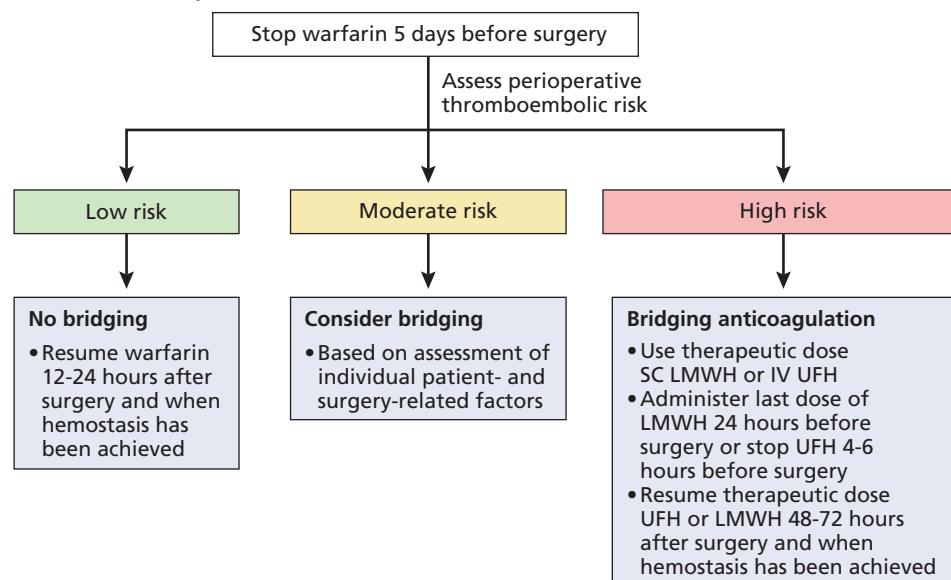
(LMWH) used for bridging. The use of these scores has not been extensively validated in the perioperative setting. Additional information about use of anticoagulants in the perioperative setting is found in Chapter 9.

In addition, the patient's prior history of bleeding, comorbidities that may affect bleeding (eg, renal function), as well as concomitant use of antiplatelet and nonsteroidal anti-inflammatory medications, are important in determining overall bleeding risk. Generally, procedures or surgeries associated with the potential for intracranial, intraocular, spinal, retroperitoneal, intrathoracic, or pericardial bleeding are considered high risk for bleeding. Procedures with a low bleeding risk include nonmajor procedures (lasting <45 minutes), such as general surgical procedures (hernia repair, cholecystectomy), dental, or cutaneous procedures. The American College of Chest Physicians (ACCP) updated guidelines on the perioperative management of antithrombotic medications in 2012 (<http://chestjournal.chestpubs.org>).

An evidence-based approach to the perioperative management of patients on warfarin undergoing major surgery is shown in Figure 2-1. Temporary discontinuation of warfarin, approximately 5 days until normalization of the INR, is recommended in all patients. Bridging anticoagulation with therapeutic-dose LMWH or unfractionated heparin (UFH) may be considered depending on the patient's risk of thromboembolism. The BRIDGE trial

randomized patients with atrial fibrillation that required warfarin interruption for a procedure or surgery to bridging anticoagulation with LMWH versus no bridging anticoagulation. The authors found that forgoing bridging anticoagulation was noninferior to perioperative bridging with LMWH for the prevention of arterial thromboembolism with the benefit of decreased major bleeding. In patients requiring minor dental procedures, warfarin may be continued with coadministration of an oral antifibrinolytic agent, if needed, or warfarin may be stopped 2 to 3 days before the procedure. Warfarin also may be continued in patients undergoing minor dermatologic procedures with the use of adjunctive local hemostatic measures as necessary. Cataract surgery also may be performed without interruption of warfarin. Perioperative anticoagulation should be used with caution after certain procedures like (i) prostate or kidney biopsy, where postoperative bleeding may be stimulated by the highly vascular tissue and endogenous urokinase; (ii) large colonic polypectomies that can be associated with bleeding at the stalk; and (iii) cardiac pacemaker or defibrillator implantation where a pocket hematoma may form. For intracranial or spinal surgery, bridging therapy is often not feasible. For patients or procedures thought to be at high risk for bleeding, an INR <1.5 should be achieved the day prior to surgery. If LMWH bridging is deemed necessary, then the last dose should be half the normal daily

Figure 2-1 Approach to perioperative management of patients on warfarin undergoing major surgery. Management should be informed by an individualized assessment of host- and surgery-related risk factors for perioperative thromboembolism and hemorrhage as well as patient values and preferences. LMWH, low-molecular weight heparin; UFH, unfractionated heparin.
Based on Douketis JD et al. *Chest*. 141:e326S.



dose and administered 24 hours before the procedure to avoid residual anticoagulant effect. Depending on the patient's underlying thrombotic risk, postoperative options include waiting 48 to 72 hours after surgery before resuming full-dose LMWH bridging therapy, using an intermediate or prophylactic LMWH, or utilizing only mechanical prophylaxis if the bleeding risk is extremely high. For neuraxial anesthesia, the dosing and timing of perioperative LMWH follow the practice guidelines laid out from the American Society of Regional Anesthesia. Like warfarin, the direct oral anticoagulants dabigatran, rivaroxaban, apixaban, and edoxaban must be discontinued before major surgery. However, unlike warfarin the predictable short half-life of these newer anticoagulants allows for relatively short-term cessation preoperatively, without the routine need for bridging anticoagulation. Most patients can safely undergo procedures within 24 to 48 hours of their last dose of these new oral anticoagulants, depending on surgical risk of bleeding. However, with renal impairment, hepatic impairment, older age, and concurrent antiplatelet medications, longer cessation intervals may be necessary preoperatively (Table 2-2). Given the short onset of action of these drugs, hemostasis must be achieved postoperatively before restarting the direct oral anticoagulants. Perioperative bridging protocols with these agents have been proposed based on pharmacokinetic data but have not been investigated systematically.

Perioperative management of antiplatelet therapy, like with oral anticoagulants, relies on an assessment of the in-

dividual patient's thrombotic risk as well as the nature of the planned procedure. In general, patients may remain on aspirin for minor dental or dermatologic procedures and cataract surgery. For major noncardiac surgery, many guidelines suggest holding aspirin for at least 7 to 10 days, though laboratory-based studies suggest sufficient aggregation response returns after 4 days without aspirin. Aspirin should be continued in patients judged to be at moderate or high risk. Patients who require coronary artery bypass grafting should remain on aspirin in the perioperative setting. If such patients are on dual antiplatelet therapy, clopidogrel or prasugrel should be held beginning 5 days before surgery.

In patients with a coronary stent who are receiving dual antiplatelet therapy and require surgery, it should be deferred, if possible, during the period of highest risk for stent thrombosis (6 weeks after placement of bare metal stents, 6 months after placement of drug-eluting stents). After this period has passed, clopidogrel or prasugrel may be suspended temporarily for surgery. If surgery cannot be delayed, dual antiplatelet therapy should be continued during and after surgery.

The direct oral anticoagulants (DOACs) apixaban, dabigatran, edoxaban, and rivaroxaban have become popular in recent years due to their stable pharmacokinetics and favorable bleeding profile. The short half-life of these agents often eliminates the need for bridging or holding for prolonged periods of time prior to invasive procedures. Idarucizumab is available as a reversal agent for dabigatran,

Table 2-2 Perioperative cessation and resumption of direct oral anticoagulants

Creatinine clearance	Type of DOAC*			
	Dabigatran		Apixaban/edoxaban/rivaroxaban	
	Bleeding risk of intervention			
	Low risk	High risk	Low risk	High risk
≥80 ml/min	≥24 h	≥48 h	≥24 h	≥48 h
50–80 ml/min	≥36 h	≥72 h	≥24 h	≥48 h
30–50 ml/min	≥48 h	≥96 h	≥24 h	≥48 h
15–30 ml/min	Not indicated	Not indicated	≥36 h	≥48 h
<15 ml/min	No official indication for use			
	There is no need for parenteral bridging with LMWH or UFH			
Resumption after procedure†	≤24 h	24–48 h	≤24 h	>48–72 h

Adapted from Heidbuchel H, Verhamme P, Alings M, et al. *Europace*. 2015;17:1467–1507, with permission of Oxford University Press (UK); © European Society of Cardiology.

DOAC, direct oral anticoagulant.

*For patients on dabigatran 150 mg twice daily, apixaban 5 mg twice daily, edoxaban 60 mg once daily, or rivaroxaban 20 mg once daily.

†Depending on whether hemostasis is achieved. If significant risk for perioperative thrombosis exists, prophylactic or intermediate doses of UFH or LMWH should be considered until full therapeutic anticoagulation with a direct oral anticoagulant is resumed.

given as a 5-g intravenous infusion. In addition, andexanet alfa is now FDA-approved to reverse the anticoagulant effect of rivaroxaban and apixaban. For most patients, holding the medication in the setting of mild bleeding is adequate given the short half-life, and antifibrinolytic agents may be used as an adjunct. For severe life-threatening bleeding, guidance documents are conflicting; however administration of a 4-factor prothrombin complex concentrate (PCCs) is often utilized. The reader is cautioned that use of 4-factor PCCs for this purpose is an off-label approach and further studies are needed to determine its efficacy and safety.

Management of perioperative hemorrhage

Perioperative hemorrhage may be due to inadequate local hemostasis or a systemic hemostatic defect. Potential hemostatic defects include an unrecognized preexisting bleeding diathesis, drugs, uremia, dilutional coagulopathy, or disseminated intravascular coagulation (DIC). Not to be overlooked is the increased risk of bleeding induced by acid-base disturbances and hypothermia. Close attention should be paid to the pattern of bleeding, specifically the timing in relation to surgery, the location, and the tempo of the bleed. A structural defect is more likely with a single site (versus multiple sites) of bleeding, with sudden onset of bleeding (versus delayed bleeding following initial hemostasis), and/or with brisk bleeding (versus slow persistent oozing).

Certain surgeries are associated with specific hemostatic defects. Excessive blood loss in patients undergoing cardiopulmonary bypass surgery may be due to the effects of the bypass circuit on platelet function and fibrinolysis or the use of antiplatelet agents, heparin, or other anti-coagulants. Liver transplantation carries unique risks due to the temporary loss of coagulation factor synthesis and enhanced fibrinolysis. During reperfusion of the transplanted liver, tissue-type plasminogen activator is released into the circulation and proteolysis of von Willebrand factor (VWF) occurs.

All patients with surgical bleeding should undergo an immediate basic hemostatic laboratory evaluation, including a platelet count, PT, aPTT, and fibrinogen. Blood must be drawn from a fresh peripheral venipuncture site due to the common contamination of blood samples with heparin flushes, saline, erythrocytes, or plasma. Significant abnormalities of any of these initial parameters suggest a systemic hemostatic defect, which may require specific hemostatic therapy. Clinically significant thrombocytopenia or fibrinogen deficiency in a bleeding surgical patient mandates appropriate therapy and further testing to identify the cause of the deficiency. In general, cryoprecipitate and platelets should be transfused to maintain a fibrinogen concentration of at least 100 mg/dL and a platelet count

of at least $50 \times 10^9/L$ ($100 \times 10^9/L$ for organ- or life-threatening bleeding), respectively. Fibrinogen concentrate is available in many centers. Hypothermia, hypocalcemia, and acid-base disturbances should be corrected. Although the thromboelastograph (TEG) has traditionally been utilized more by the anesthesiologist than the hematologist, this test can provide an accurate and rapid method of diagnosing hyperfibrinolysis, as seen in cardiopulmonary bypass and orthotopic liver transplant. There is growing interest in the utilization of TEG to guide transfusion replacement therapy in trauma-induced coagulopathy and in surgical patients to predict thromboembolic events. For more discussion about TEG, see Chapter 12.

If basic hemostatic laboratory parameters are normal or bleeding persists after correction of these parameters, inadequate local hemostasis due to vessel injury is suggested and surgical reexploration should be considered. Some systemic bleeding diatheses (such as mild deficiency of factors VIII, IX, or XI; von Willebrand disease; qualitative platelet defects; or a disorder of fibrinolysis) may not be identified by basic laboratory testing. Patients with mild factor XI deficiency, for example, may have a normal or near-normal aPTT. Clinicians should maintain a high index of suspicion for these disorders in a patient with persistent unexplained surgical bleeding and test for specific coagulation factor levels as indicated.

Adjunctive agents may be used alone for minor bleeding or as a complement to product replacement for major bleeding in selected patients and clinical circumstances. DDAVP (desmopressin acetate) may be used for mild bleeding in patients with mild hemophilia A, mild von Willebrand disease, or a qualitative platelet defect. Ideally, response to this agent should be documented before its use in the acute setting. Mucocutaneous bleeding may respond to antifibrinolytic therapy with tranexamic acid or ϵ -aminocaproic acid. Oral or intravenous conjugated estrogens, given for 5 to 7 days preoperatively, may decrease platelet-related bleeding in patients with chronic kidney disease. Topical fibrin sealants may be used to reinforce local hemostasis in patients with underlying bleeding disorders.

Hemostatic agents have been used to prevent or treat surgical bleeding in patients without known hemostatic disorders. Tranexamic acid and ϵ -aminocaproic acid have been shown to reduce blood loss and blood transfusion after cardiac surgery, liver transplantation, orthopedic surgery, and prostatectomy. An observational study of 4,374 patients undergoing coronary revascularization surgery on cardiopulmonary bypass showed that use of these agents was associated with a 30% to 40% reduction in surgical blood loss without an increased risk of thromboembolism.

Recombinant factor VIIa (rFVIIa) is approved in the United States for the treatment of patients with congenital hemophilia A or B with inhibitors, patients with acquired hemophilia, congenital factor VII deficiency, and Glanzmann thrombasthenia with platelet refractoriness. Despite these limited indications, the majority of rFVIIa usage is off-label, especially for the management of perioperative bleeding. Controlled trials have shown rVIIa to be of no benefit in reducing transfusion in cirrhotic patients undergoing partial hepatectomy or orthotopic liver transplantation. Recent studies have highlighted the potential thrombotic risk with off-label use of rVIIa. In a meta-analysis of 35 randomized controlled trials of rVIIa for unapproved indications, the overall rate of thromboembolism in rVIIa-treated subjects was 9.0%. The rate of arterial, but not venous, events was higher in subjects receiving rVIIa, particularly among those 65 years and older. The indiscriminate use of rVIIa for the management of perioperative hemorrhage should be discouraged; however, it may be useful for selected patients with life-threatening bleeding despite conventional measures and appropriate transfusion therapy. Advanced age and preexisting cardiovascular risk factors may increase the risk of arterial thromboembolic complications with rVIIa.

Prothrombin complex concentrates (PCCs) are plasma-derived concentrates of the vitamin K-dependent clotting factors. PCCs are classified as either 3-factor or 4-factor depending on the amount of FVII included. These products are approved for the treatment of hemophilia B. The 4-factor PCC Kcentra is approved for the urgent reversal of acquired coagulation factor deficiency induced by vitamin K-antagonist therapy in adult patients with acute major bleeding, and work is ongoing to determine its efficacy in reversal of the direct oral anticoagulants. Activated PCCs (APCCs) contain variable amounts of activated vitamin K-dependent clotting factors and are indicated for the treatment of patients with hemophilia and inhibitors. The use of PCCs and APCCs for the management of perioperative hemorrhage has been reported but not prospectively investigated. Further studies are needed before use of these agents to control surgical bleeding can be recommended.

Fibrin sealant, also known as fibrin glue, consists of 2 main components: human fibrinogen and human thrombin. When delivered together, the thrombin cleaves fibrinogen to form a stable fibrin clot on the tissue surface. Although randomized clinical trial data and evidence-based guidelines are lacking, fibrin sealant is used for hemostasis in cardiac and thoracic surgery, trauma, liver and spleen lacerations, and dental procedures. It also has been used on the liver surface following orthotopic liver trans-

plantation to augment local hemostasis. Fibrin glue is also utilized as an adhesive to seal dural leaks, repair otic ossicles and bony defects, and provide adhesion for skin grafts. Fibrin glue products are safe and effective, but rare side effects include hypotension, anaphylaxis, infection transmission, and air embolism. With the substitution of human-derived thrombin for bovine-derived product in fibrin glue formulations, the previously reported bleeding diatheses associated with antibovine factor V or factor II antibodies (bovine factor V is a contaminant of bovine thrombin preparations) that cross-react with endogenous factor V/II no longer occur.

Prevention and treatment of postoperative venous thromboembolism

VTE is a common and potentially lethal complication of surgery. Pulmonary embolism remains the leading cause of preventable death in hospitalized patients. Despite contemporary thromboprophylaxis, postoperative VTE rates remain unacceptably high, leading the Agency for Health Care Research and Quality to cite prevention of VTE as the number one priority for improving patient safety in hospitals.

Risk factors for VTE in surgical patients include type and extent of surgery or trauma, general anesthesia for greater than 30 minutes, longer duration of hospitalization, advanced age, cancer, personal or family history of VTE, obesity, immobility, infection, presence of a central venous catheter, pregnancy or the postpartum state, and thrombophilia. Several prediction models have been developed to estimate VTE risk in surgical patients, but all have important limitations. A general risk stratification schema recommended by the ACCP for patients undergoing non-orthopedic surgery is shown in Table 2-3. The ACCP guidelines utilize 2 validated risk stratification models based on risk-factor point systems (Rogers score and Caprini score). These scoring systems estimate an individual's perioperative VTE risk as low, moderate, or high by assigning a point value for various patient- and procedure-related risk factors (eg, age, obesity, degree of immobility, specific comorbidities, type of surgery planned, known thrombophilia). In general, very low-risk (<0.5%) and low-risk (~1.5%) patients tend to be younger than 40 years old, have no adverse patient- or surgery-related risk factors, and require general anesthesia for less than 30 minutes. Patients in the moderate risk (~3.0%) category include those with risk factors who are undergoing minor surgery and those age 40 to 60 years who have no additional surgery- or patient-related risk factors, but who will require general anesthesia for >30 minutes. High-risk patients generally include individuals >60 years of age undergoing major

Table 2-3 General VTE risk stratification for patients undergoing nonorthopedic surgery

Risk category	Risk of VTE (without prophylaxis)	Type of surgery				General thromboprophylaxis strategies
		Major general, thoracic, or vascular	Gastrointestinal, urological, vascular, breast, or thyroid	Plastic and reconstructive	Other surgical populations	
Very low	<0.5%	Rogers score <7	Caprini score 0	Caprini score 0–2	Most outpatient or same-day surgery	Early ambulation
Low	~1.5%	Rogers score 7–10	Caprini score 1–2	Caprini score 3–4	Spinal surgery for nonmalignant disease	Mechanical prophylaxis, preferably with IPC
Moderate	~3.0%	Rogers score >10	Caprini score 3–4	Caprini score 5–6	Gynecologic non-cancer surgery Cardiac surgery Most thoracic surgery Spinal surgery for malignant disease	Pharmacologic or mechanical prophylaxis
High	~6.0%	NA	Caprini score ≥5	Caprini score 7–8	Bariatric surgery Gynecologic cancer surgery Pneumonectomy Craniotomy Traumatic brain injury Spinal cord injury Other major trauma	Combination of pharmacologic and mechanical prophylaxis

Adapted from ACCP guidelines.

IPC, intermittent pneumatic compression; VTE, venous thromboembolism; NA, not applicable.

surgery, as well as those age 40 to 60 years with additional risk factors who will be having major surgery.

A strategy for thromboprophylaxis should be based on the estimated risk of VTE and bleeding and the type of surgery. Prophylactic measures include early ambulation; lower extremity intermittent pneumatic compression (IPC); graduated compression stockings (GCS); and pharmacologic prophylaxis with low dose UFH, LMWH, fondaparinux, or oral anticoagulation and are outlined in Table 2-3. In patients judged to be at high risk for bleeding, mechanical prophylaxis is favored over pharmacologic strategies unless and until bleeding risk diminishes. Surveillance compression ultrasonography to screen for DVT and inferior vena cava filter insertion for primary prevention of DVT are generally not recommended in surgical patients.

In the absence of a heightened bleeding risk, most patients undergoing major orthopedic surgery should receive pharmacologic thromboprophylaxis. LMWH, fondaparinux, low-dose UFH, warfarin, and aspirin (all GRADE 1B) are all reasonable options for patients undergoing hip fracture surgery. Any of these agents, as well

as the direct oral anticoagulants, dabigatran, apixaban, and rivaroxaban (all GRADE 1B), may be used following total hip or total knee arthroplasty. IPC is also reasonable in combination with pharmacologic prophylaxis during the hospital stay or in lieu of pharmacologic prophylaxis, particularly in patients at increased risk for bleeding. Pharmacologic prophylaxis should be continued for a minimum of 10 to 14 days after major orthopedic surgery. Extended prophylaxis for 4 to 5 weeks should be considered after major orthopedic surgery and major abdominal or pelvic surgery for cancer. A recent multicenter randomized controlled trial evaluating thromboprophylaxis after total hip or knee arthroplasty found that VTE rates after 5 days of rivaroxaban followed by aspirin for either 9 days (knee) or 30 days (hip) was not significantly different from continued use of rivaroxaban. Clinical practice guidelines are currently being updated to incorporate these data, but the results are encouraging.

The timing of initiation of prophylaxis varies based on the procedure and regional practice patterns. In Europe, LMWH is usually started at half doses 12 hours before

surgery, whereas in the United States, it is common to start full doses 12 to 24 hours after surgery. Bleeding rates are low with both strategies and are greater when LMWH is started within 4 hours before or after surgery. Prophylactic warfarin begun just before or immediately after surgery is less commonly associated with hemorrhage, but it is also less effective in preventing DVT. LMWH, fondaparinux, dabigatran, apixaban, rivaroxaban, and edoxaban should be avoided in patients with renal failure. Postoperative bleeding risk is often best estimated by the surgeon and discussion about the patient-specific prophylaxis plan must be made in collaboration with the surgical team and patient.

When VTE occurs postoperatively, the consultant may be asked for treatment recommendations. For most low-risk procedures, full anticoagulation can be initiated safely within 12 to 24 hours after surgery. The agent of choice in the immediate postoperative period is continuous-infusion UFH because of its short half-life and rapid reversibility with protamine if bleeding develops. Contraindications to immediate postoperative anticoagulation include active bleeding and certain neurosurgical or ophthalmologic procedures in which bleeding would risk permanent injury. In patients with postoperative VTE and a contraindication to anticoagulation, insertion of a retrievable inferior vena cava filter may be required. Once it is deemed to be safe, anticoagulation should be initiated and a plan made for retrieval of the filter. The duration of anticoagulation after a first, uncomplicated postoperative VTE is generally 3 months. Longer treatment may be indicated for recurrent VTE and in the setting of certain hypercoagulable conditions, such as active cancer or APLS.

- The type of postoperative thromboprophylaxis required depends on the patient's risk of VTE, the type of surgery, and the patient's risk of bleeding.
- Management of acute VTE in a postoperative patient is similar to the approach in a nonsurgical patient; however, the risk of postoperative bleeding with systemic therapeutic anticoagulation must be carefully considered.

Common inpatient consultations

This section focuses on 2 common hematological consultations in hospitalized patients: thrombocytopenia and anemia.

Thrombocytopenia

Thrombocytopenia, defined as a platelet count less than $150 \times 10^9/L$, is one of the most common reasons for hematology consultation in the hospitalized patient. In a registry of >64,000 patients admitted to the hospital with acute coronary syndromes, 6.8% had thrombocytopenia at baseline and 13% developed it during their hospital stay. In a study of 2,420 hospitalized medical patients receiving heparin for at least 4 days, 36% developed thrombocytopenia. A systematic review of 6,894 critically ill patients reported that thrombocytopenia occurred in 8% to 68% of patients on admission to the intensive care unit (ICU) and developed in 13% to 44% during their ICU stay. The main challenges in the management of hospitalized patients with thrombocytopenia are to identify the underlying cause and recognize when urgent intervention is required.

A traditional approach to thrombocytopenia is to classify etiologies into conditions of decreased platelet production, increased platelet destruction, or sequestration. Although this framework is comprehensive, it does not consider features related to the individual patient. Furthermore, many disorders have more than one mechanism of thrombocytopenia (eg, immune thrombocytopenia [ITP] may be caused by both platelet destruction and platelet underproduction), and some critically ill patients may also have more than one cause. We propose the following practical approach to the diagnosis of thrombocytopenia in the hospitalized patient tailored to specific elements of the history, physical examination, and laboratory investigations (Figure 2-2): (1) exclude thrombocytopenic emergencies, (2) examine the blood film, (3) consider the clinical context, (4) assess the degree of thrombocytopenia, (5) establish the timing of thrombocytopenia, and (6) assess the patient for signs of bleeding and/or thrombosis.

KEY POINTS

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- Surgical bleeding risk is associated with both patient- and surgery-related factors. Patient factors include the presence of an underlying congenital or acquired hemostatic defect and use of drugs that affect hemostasis. Surgical factors include the nature and extent of the intervention, the vascularity and fibrinolytic activity of the surgical bed, the compressibility of the site, and the ability to achieve surgical hemostasis.
 - A focused medical history is the most important tool to assess the risk of surgical bleeding.
 - Perioperative management of patients receiving antiplatelet or anticoagulant drugs depends on the patient's risk of thromboembolism and the risk of surgical bleeding.
 - Since apheresis for both hematologic and nonhematologic conditions is a common consult, hematologists should be aware of the evidence-based indications for this procedure.

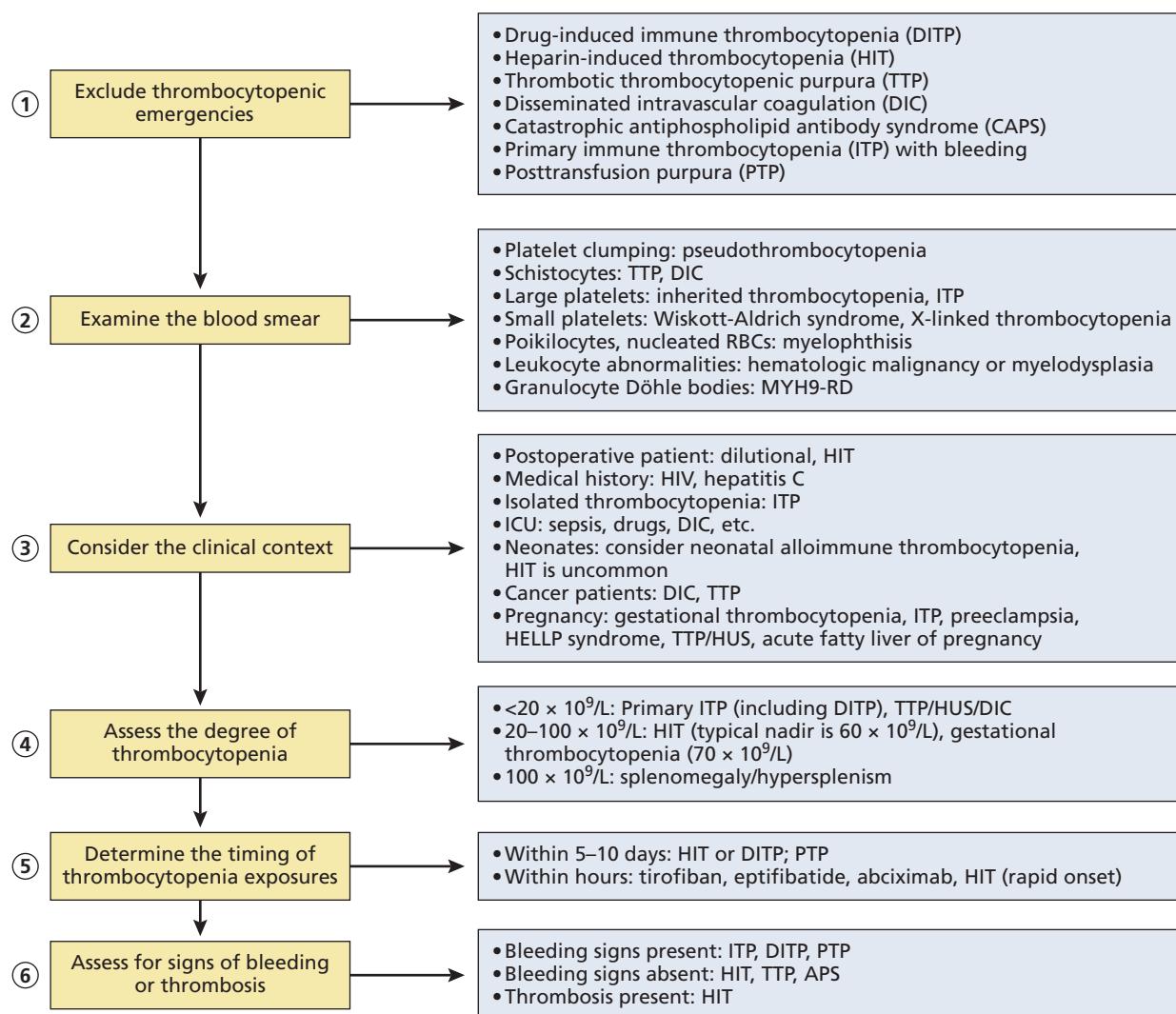


Figure 2-2 Practical approach to the patient with thrombocytopenia. Adapted with permission from Arnold DM, Lim W. *Semin Hematol*. 2011;48:251–258.

1. Exclude thrombocytopenic emergencies

CLINICAL CASE

A 62-year-old man is in the ICU following complications from cardiac surgery. You are consulted on postoperative day 6 because his platelet count is $30 \times 10^9/\text{L}$. His left leg is swollen, and one patch of skin around his left ankle is gangrenous. His PT, aPTT, and fibrinogen are normal. Based on the 4Ts score—a clinical prediction rule to estimate the pretest probability of heparin-induced thrombocytopenia (HIT)—you decide he has a high probability of HIT and recommend changing all anticoagulation to nonheparin products and sending specific HIT testing.

Any thrombocytopenic condition could become an emergency if it is severe and serious bleeding occurs (eg, intracranial hemorrhage), but some thrombocytopenic disorders are emergencies in themselves regardless of the degree of thrombocytopenia because of their associated risk of significant morbidity and mortality if not promptly recognized and managed. These include drug-induced immune thrombocytopenia (DITP), HIT, thrombotic thrombocytopenic purpura (TTP), sepsis and DIC, catastrophic antiphospholipid antibody syndrome (CAPS), and posttransfusion purpura (PTP). These diagnoses should be considered initially for any patient with thrombocytopenia. Consideration of the peripheral blood film and other laboratory values, clinical and medication history, and timing of thrombocytopenia can help with early recognition and treatment.

Drug-induced immune thrombocytopenia and heparin-induced thrombocytopenia

DITP is characterized by severe thrombocytopenia and may be associated with serious bleeding complications. It is usually an idiosyncratic reaction caused by drug-dependent platelet-reactive antibodies that cause rapid platelet clearance (see Chapter 11). An expanded list of drugs and the level of evidence for their association with thrombocytopenia has been reported online (<http://www.ouhsc.edu/platelets>), but it is imperative to ask the patient about not just prescribed medications but over-the-counter medications and herbal medications as these may also be associated with thrombocytopenia. Classic DITP reactions, such as quinine-induced DITP, result in thrombocytopenia that occurs 5 to 10 days after first exposure to the drug, whereas the glycoprotein IIb/IIIa inhibitors abciximab and eptifibatide can cause thrombocytopenia within hours of the first drug exposure. Withdrawal of the offending drug is often enough to allow platelet count recovery, but intravenous immunoglobulin (IVIG) may be needed in severe cases of DITP.

HIT is a distinct clinical syndrome associated with thrombosis rather than bleeding. The prevalence is estimated to be between 0.1% and 5.0%. HIT should be considered in patients with use of heparin who develop new onset of thrombocytopenia or thrombosis. Classically, patients present within 5 to 10 days of heparin exposure; however, HIT can occur more rapidly (<1 day) in patients who may have had heparin exposure in the preceding 30 to 100 days. The risk of HIT is highest with unfractionated heparin and less with LMWH. Further, the risk of HIT is higher with therapeutic doses of UFH or LMWH compared with subcutaneous prophylactic doses. The 4Ts probability scale can be used to assess the likelihood of having HIT. With this scale, the degree and timing of thrombocytopenia, presence of thrombosis, and possible alternative causes of thrombocytopenia are each independently considered on a scale of 0 to 2 and then summed together. A low score of 0 to 3 indicates a <1% probability of HIT, an intermediate score of 4 to 5 indicates an approximate 10% probability of HIT, whereas a high score of 6 to 8 is associated with an approximate 50% probability of HIT. The diagnosis of HIT can be confirmed with either antigen or functional assays; however, the ASH Choosing Wisely campaign recommends against testing or treating for suspected HIT in patients with a low pretest probability score. Antigen assays for anti-PF4-heparin antibodies lack specificity and may lead to false-positive results in critically ill patients and functional platelet-activation tests, such as the serotonin release assay, should be used to confirm the diagnosis. Treatment of

patients with suspected or confirmed HIT requires anti-coagulation with a nonheparin alternative such as a direct thrombin inhibitor. The direct oral anticoagulants may be an attractive option, but their efficacy needs to be prospectively evaluated prior to widespread use. While case reports have implicated fondaparinux in the development of HIT, it has been used successfully to treat HIT in a variety of patients as well. Without proper treatment, up to 55% of patients develop thrombosis, and approximately 5% to 10% of patients will die as a result of thrombotic complications.

Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome (HUS)

TTP and hemolytic uremic syndrome (HUS) are thrombotic microangiopathies characterized by microangiopathic hemolytic anemia and thrombocytopenia. These disorders should be considered in any patient with anemia, thrombocytopenia, and schistocytes on peripheral blood film in the absence of another identifiable cause such as DIC. The clinical manifestations of these disorders overlap; however, patients with TTP often have neurological complications, whereas renal impairment predominates in HUS (see Chapter 11). TTP results from either a congenital deficiency of ADAMTS13 (*a disintegrin and metalloproteinase with a thrombospondin type 1 motif*), the VWF-cleaving protease, or an acquired antibody which can be either neutralizing or nonneutralizing against ADAMTS13 activity. ADAMTS13 activity is typically <10% in patients with TTP. While testing of both ADAMTS13 levels and antibodies is available, treatment should not be withheld while awaiting results if suspicion is high. With proper treatment, survival of TTP patients is 85%; however, without it survival drops to 10%. While scores have been created to predict the likelihood of ADAMTS13 activity <10%, prospective studies in which patient management is based on such scores have not been published. Management requires prompt initiation of daily therapeutic plasma exchange with 1.0 to 1.5 plasma volumes in conjunction with corticosteroids if the TTP is thought to be due to an acquired inhibitor of ADAMTS13. If plasma exchange cannot be initiated, then infusion of fresh frozen plasma (FFP) may be given until such time that plasma exchange can occur. In patients with congenital ADAMTS13 deficiency or in those with acquired ADAMTS13 inhibition in whom plasma exchange cannot be initiated promptly, infusion of FFP to replace ADAMTS13 should be initiated. A major role of the consultant is to ensure coordination of care with multiple services that are often involved in the management of TTP patients, including the intensive care unit, surgical or radiology ser-

vices placing appropriate catheters for plasma exchange, and the blood bank.

Remission in TTP can be defined as either clinical or laboratory. Some patients may experience a clinical remission but continue to have reduced levels of ADAMTS13 and/or detectable antibodies. Approximately 35% of patients experience a relapse following plasma exchange. Relapse rates are highest among patients with persistent ADAMTS13 levels of <10%, males, and during the first year of remission. In acquired TTP patients who are refractory or relapse despite plasma exchange, rituximab may induce remission and subsequent cessation of plasma exchange. In patients with congenital TTP and reaction to plasma, administration of a plasma-derived factor VIII product has been successful in decreasing TTP events. A recombinant ADAMTS13 product is in development.

Plasma exchange usually does not provide benefit to patients with HUS. The most common form of HUS is associated with bloody diarrhea and is caused by enteric infection with strains of *Escherichia coli* that produce Shiga-like toxins (typical HUS or diarrhea-positive HUS). This variant accounts for up to 95% of all HUS in children, often occurs in epidemics, and generally is self-limited. *Streptococcus pneumoniae*-associated HUS accounts for 5% to 15% of all childhood HUS cases and is due to the exposure of the Thomsen-Freidenreich cryptantigen on the surface of cell membranes by neuraminidase produced by the bacteria. It usually occurs in the setting of pneumonia and empyema, with a lesser association with meningitis. The disease has a higher mortality and more long-term morbidity than *E. coli*-associated HUS. Renal failure appears to be a more prominent feature in those with HUS as compared to TTP. The atypical form of HUS occurs without a diarrheal prodrome (diarrhea-negative HUS) and is associated with a higher incidence of end-stage kidney disease and mortality. This form occurs more commonly in adults and often is caused by a dysregulation of the complement system. Mutations in genes encoding complement proteins, including factor H, membrane cofactor protein (CD46), factor I, and factors B and C3, have been described. Like TTP, management of atypical HUS often starts with empiric initiation of plasma exchange. Complement inhibition with the monoclonal antibody eculizumab, which targets C5, may improve renal function and hematologic parameters while allowing for discontinuation of plasma exchange in patients with atypical HUS. Whether eculizumab therapy is lifelong or may be stopped during remission is unknown and under active investigation. Patients on eculizumab therapy should receive meningococcal vaccination at least 2 weeks prior to initiating therapy, and those who must start comple-

ment inhibitor therapy prior to this should be considered for prophylactic antibiotics.

Disseminated intravascular coagulation and sepsis

DIC occurs in critically ill patients in the setting of a serious underlying disease, such as sepsis, classical meningo-coccemia, trauma, malignancy, and pregnancy catastrophes, including placental abruption and amniotic fluid embolism. DIC also may complicate poisoning, major hemolytic transfusion reactions, and severe HIT. DIC is caused by enhanced thrombin generation because of an imbalance in the normal procoagulant and anticoagulant pathways and results in a microangiopathic hemolytic anemia. As a result, many patients develop significant thrombotic complications, including peripheral ischemia and skin gangrene. The clinical features are variable, and numerous tests of hemostasis become abnormal, including thrombocytopenia, increased fibrin degradation products such as D-dimers, prolongation of the PT and aPTT, decreased fibrinogen concentration, and decreased protein C concentration. The peripheral blood smear will often show schistocytes. A significant reduction in the level of fibrinogen may indicate early or subclinical DIC even if it does not result in fibrinogen levels below laboratory reference intervals. DIC is a dynamic process requiring repeated measurements of hemostasis and careful clinical monitoring. DIC may result in significant bleeding and may be the presenting feature of a hematologic malignancy such as acute promyelocytic leukemia. Early initiation of therapy may help arrest the coagulopathy. For more details, see Chapter 20.

Guidelines and consensus statements for the management of DIC highlight the importance of treating the underlying condition even though this can be challenging. While evidence is lacking to clearly guide the use of prophylactic platelet transfusions, they should generally be reserved for patients with a platelet count below $50 \times 10^9/L$, those at high risk of bleeding, or patients with worsening thrombocytopenia. Similarly, plasma transfusions are primarily reserved for patients with an increased PT and bleeding, and cryoprecipitate or fibrinogen concentrates are indicated for patients with severe hypofibrinogenemia (fibrinogen <100 mg/dL). Correction of the fibrinogen deficit will often lead to adequate correction of the PT and aPTT without further plasma infusion. Prophylactic doses of UFH or LMWH are recommended for prevention of venous thromboembolism, and therapeutic doses should be considered for patients with thrombotic complications such as venous or arterial thrombosis, severe purpura fulminans, or vascular skin infarctions. Several coagulation factor concentrates have been investigated for the treatment of severe sepsis and DIC.

Catastrophic antiphospholipid antibody syndrome

CAPS occurs in <1% of patients with the antiphospholipid antibody syndrome. It is a life-threatening condition that requires prompt recognition and management. Diagnostic criteria for CAPS are: (i) involvement of 3 or more organs, systems, or tissues; (ii) development of symptoms simultaneously or in <1 week; (iii) confirmation by histopathology of small vessel occlusion in at least 1 organ or tissue; and (iv) laboratory confirmation of the presence of antiphospholipid antibodies (lupus anticoagulant; or anti-cardiolipin or anti- β -2-glycoprotein 1 antibodies). A registry of patients with CAPS has provided important information on diagnosis and management (https://ontocrf.grupocostaisa.com/es_ES/web/caps/home). Infection is the most commonly identified precipitant, but other triggers such as trauma, withdrawal of anticoagulation, and neoplasia have also been described. Approximately 40% of patients with CAPS have no obvious underlying cause and mortality often exceeds 50%. Treatment consists of plasma exchange in addition to aggressive therapy such as anticoagulation, corticosteroids, and IVIG. This multimodality approach is supported by data from the “CAPS Registry” with use of multiple agents being reported in the management; anticoagulation (87%), corticosteroids (86%), cyclophosphamide (36%), IVIG (22%), and antiplatelet agents (10%). Rituximab has also been used with some success in more refractory cases.

Posttransfusion purpura

PTP is a syndrome characterized by severe thrombocytopenia and bleeding that develops 7 to 10 days after the transfusion of any platelet-containing blood product (such as platelet or red blood cell [RBC] concentrates). It typically affects women who have had a previous pregnancy or blood transfusion and most commonly is due to antibodies against human platelet antigen 1a (HPA-1a). The incidence of PTP is estimated at 1 to 2 per 100,000 transfusions, and it appears to be less common with leukocyte-reduced blood products. The pathophysiology remains uncertain, but may involve the formation of immune complexes, adsorption of soluble platelet antigens onto autologous platelets, or the induction of platelet autoantibodies. Diagnosis involves recognizing thrombocytopenia that occurs after transfusion of platelet-containing products and demonstrating circulating alloantibody to HPA-1a antigen in a patient whose own platelets lack this antigen. IVIG has been used to successfully treat PTP. Patients with PTP who require additional transfusions for bleeding or severe thrombocytopenia should receive HPA-1a-negative blood products if available.

2. Examine the blood film

Examination of the blood film is necessary for all patients with thrombocytopenia. Platelet clumps are suggestive of pseudothrombocytopenia, a laboratory artifact caused by naturally occurring antibodies directed against the anticoagulant ethylenediaminetetraacetic acid (EDTA). A repeat sample collected in citrate or heparin tube usually resolves the platelet clumping. The size and morphology of the platelets can be assessed, and findings such as large platelets can indicate a state of high platelet turnover, such as ITP. The blood film also allows for morphological assessment of erythrocytes and leukocytes, which may provide important clues to the underlying diagnosis: the presence of schistocytes raises the possibility of a microangiopathic process such as TTP or DIC; poikilocytes or nucleated RBCs may reflect a myelophthisic process; abnormal leukocytes may indicate a hematologic malignancy or myelodysplasia; toxic granulation of neutrophils is seen in sepsis; and neutrophilic inclusions known as Döhle bodies are associated with hereditary forms of thrombocytopenia, such as the MYH9-related disorders (MYH9-RD).

3. Consider the clinical context

The clinical context in which the thrombocytopenia developed is an important clue to the underlying diagnosis. Medical history may reveal a source for thrombocytopenia such as medications, liver disease, or secondary ITP such as HIV or hepatitis C virus (HCV). Thrombocytopenia is a common occurrence among critically ill patients, particularly those with underlying malignancies prone to DIC or thrombotic microangiopathies. Age also helps narrow the differential diagnosis; for example, neonatal alloimmune thrombocytopenia (NAIT) should be suspected in any newborn with severe unexpected thrombocytopenia, and HIT is distinctly rare in children. Thrombocytopenia during pregnancy should lead to consideration of gestational thrombocytopenia, ITP, or more severe conditions such as preeclampsia, HELLP syndrome, TTP/HUS, or acute fatty liver of pregnancy.

Thrombocytopenia in patients admitted to the ICU

Approximately 40% of critically ill patients have thrombocytopenia; however, the frequency varies based on case mix and most thrombocytopenia in the ICU is due to multifactorial causes. In a systematic review of medical, surgical, and mixed ICU studies, prevalent thrombocytopenia (on ICU admission) occurred in 8.8% to 67.6% of patients, and incidental thrombocytopenia (during ICU stay) occurred in 13.1% to 44.1% of patients. Thrombocytopenia was an independent risk factor for mortality. The association between thrombocytopenia and bleeding remains un-

certain in this population and is likely based on additional patient factors.

Heparin-induced thrombocytopenia in the ICU

The frequency of HIT in ICU patients is 0.3% to 0.5%, which represents roughly 1 in 100 patients with thrombocytopenia in this setting; thus, HIT is uncommon in this population. The diagnosis and management of HIT in critically ill patients can be challenging. After major surgery, a rapid decline in platelet count beginning on days 1 to 3 is expected; in contrast, thrombocytopenia that begins between days 5 and 14, or the development of new thrombosis in an already thrombocytopenic patient may indicate HIT. An expanded discussion on HIT can be found in the section above and in Chapter 11.

Immune thrombocytopenia

Severe isolated thrombocytopenia in an otherwise well individual may represent ITP. Many patients with ITP will have minimal bleeding despite significant thrombocytopenia. Additional risk factors such as patient age, comorbidities, and medications may increase an individual patient's risk of bleeding. First-line therapy for adults with ITP is a course of corticosteroids and IVIG may be used if a rapid platelet count is needed, such as in the setting of life-threatening bleeding. Pulse dexamethasone appears to have similar efficacy to a prolonged taper of prednisone, with lower incidence of adverse events. For those failing first-line therapy, additional treatment options include splenectomy, rituximab, and thrombopoietin receptor agonists. Overall treatment should be aimed at reducing bleeding symptoms and improving health-related quality of life. The ASH Choosing Wisely campaign recommends no treatment for adults with ITP in the absence of bleeding or a very low platelet count, usually defined as $<30 \times 10^9/L$, in order to avoid the unnecessary cost and side effects associated with treatment. The decision to treat should be individualized for each patient and account for the patient's symptoms, additional risk factors for bleeding, social factors such as distance from the hospital, side effects of possible therapy, any upcoming procedures, and patient preferences. Clinical guidelines for the treatment of ITP have been previously published and undergo periodic review, so the most updated published guidelines should be consulted for specifics of current treatment recommendations.

4. Consider the severity of thrombocytopenia

The severity of thrombocytopenia is an important clue to the diagnosis. Significant thrombocytopenia, defined as platelet counts $<20 \times 10^9/L$ is typical of primary or sec-

ondary ITP, DITP, and microangiopathic processes such as TTP/HUS and DIC. HIT generally causes a median platelet count nadir of $60 \times 10^9/L$; whereas mild thrombocytopenia can be the result of splenomegaly, primary bone marrow failure, and congenital thrombocytopenias. In patients with sepsis, platelet counts are variable but thrombocytopenia tends to be mild or moderate. Gestational thrombocytopenia typically presents with platelet counts of greater than $70 \times 10^9/L$, which often helps distinguish it from ITP in pregnancy.

5. Establish the timing of onset of thrombocytopenia

The documentation of a normal platelet count before the acute illness is helpful in narrowing the cause of thrombocytopenia. A search for exposures to drugs or blood transfusion is important. Immune-mediated platelet disorders, including classic HIT, DITP, and PTP, typically occur 5 to 10 days after exposure; however, certain drugs such as tirofiban, eptifibatide, or abciximab may cause thrombocytopenia within hours of first exposure. Rapid-onset HIT can occur after re-exposure to heparin when platelet-reactive antibodies are already present, and delayed-onset HIT is characterized by thrombocytopenia and thrombosis occurring several weeks after heparin exposure.

6. Assess for signs of bleeding and/or thrombosis

Typical platelet-type bleeding presents as petechiae or bruising; oral petechiae or purpura; and gastrointestinal, genitourinary, or intracerebral hemorrhage. Bleeding is common in patients with DITP, severe primary ITP, and in newborns with NAIT. Despite the presence of thrombocytopenia, however, bleeding is rare in HIT and TTP, because these are predominantly prothrombotic disorders and therefore the findings of thrombosis may be more diagnostic.

Anemia

Perioperative transfusion and ICU setting

Anemia is common in hospitalized patients, especially in the ICU and the perioperative setting. Approximately 25% to 30% of patients in the ICU will have a hemoglobin (Hb) level $<9 \text{ g/dL}$ and approximately one-third of critically ill patients will receive an RBC transfusion at some point during their ICU stay. Over the past decade, considerable debate has centered on the role of RBC transfusion in critically ill and perioperative patients, largely based on the realization that transfusion may be associated with an increase in infectious risk, postoperative complications, and overall mortality. Therefore, the threshold for RBC transfusion in ICU and surgical patients has changed over time.

A landmark trial investigating the benefit of a liberal or restrictive transfusion strategy in the ICU was the Transfusion Requirements in Critical Care trial. In this trial, 838 critically ill patients with hemoglobin values <9 g/dL were randomized to a transfusion strategy that maintained hemoglobin concentrations between 10 and 12 g/dL or a transfusion strategy that maintained hemoglobin concentration between 7 and 10 g/dL. Overall, there was no significant difference in 30-day mortality; however, in-hospital mortality was significantly lower in the restrictive strategy group. This study suggests that RBC transfusion is generally not required for hemoglobin concentrations >7 g/dL in the ICU. The findings of a 2012 Cochrane meta-analysis show that, in general, a liberal RBC transfusion strategy (transfusion for <10 g/dL) compared with a restrictive strategy (transfusion for Hb 7 to 8 g/dL) does not improve clinical outcomes, and a restrictive transfusion strategy is as safe, if not safer. While different guidelines have recommended that transfusion is not indicated for Hb >10 g/dL, the lower threshold varies from 6 to 8 g/dL. The American Association of Blood Banks guidelines recommend that in hemodynamically stable patients without active bleeding, a transfusion threshold of 7 to 8 g/dL should be adopted. In certain high-risk populations (eg, preexisting cardiovascular disease) or those with symptoms of chest pain, orthostatic hypotension, tachycardia unresponsive to fluid resuscitation, or congestive heart failure, transfusion should be considered at hemoglobin concentrations of <8 g/dL. The Transfusion Requirements in Septic Shock trial showed that a threshold of 7 g/dL compared to 9 g/dL was as safe in patients with septic shock. In postoperative surgical patients, including those with stable cardiovascular disease, transfusion should be considered at a hemoglobin concentration of <7 to 8 g/dL. In hospitalized stable patients with acute coronary syndrome, evidence is lacking on the optimal transfusion strategy, although some experts suggest transfusion for Hb <8 g/dL and consideration of transfusion between 8 and 10 g/dL. Use of erythropoiesis-stimulating agents such as recombinant human erythropoietin (rhEpo) have been used with varying success in the ICU. While some studies have shown a decrease in transfusions in patients receiving rhEpo for anemia in the critical care setting, other studies have not shown a benefit but did demonstrate increased rates of thromboembolic events. At this time, routine use of rhEpo should not be considered in this patient population, but there may be individual patients for which its use may be beneficial after consideration of the risks and benefits.

Several subsequent studies have supported these observations, and recent surveys suggest that transfusion practices have changed toward a more restrictive approach. The de-

cision to transfuse should be based on an individualized assessment of the patient's clinical status, oxygen delivery needs, and the pace of fall in hemoglobin rather than on a predetermined hemoglobin trigger. Accordingly, the ASH Choosing Wisely campaign recommends transfusion of the smallest effective dose to relieve symptoms of anemia or to restore the patient to a safe hemoglobin range.

Most RBC transfusions administered in the perioperative setting are allogeneic. Autologous RBCs, collected through preoperative autologous donation (PAD) or intraoperative blood salvage, remain an option for some patients. However, due to increased costs, risk of bacterial growth during liquid storage, volume overload, hemolysis from improper handling of stored units, lack of benefit with regard to decreased overall transfusion requirements, and clerical error resulting in inadvertent administration of an allogeneic product, it is recommended that PAD be restricted to healthy individuals requiring blood-intensive surgeries in which the likelihood of blood loss in excess of 500 to 1,000 mL is at least 5% to 10%.

Refusal of blood

Not uncommonly, hematologists are asked to provide consultation for patients who refuse blood transfusions (eg, Jehovah's Witnesses), including autologous blood transfusions. Most Jehovah's Witnesses do not accept any of the 4 major components of whole blood (ie, red blood cells, platelets, plasma, and white blood cells), but decisions regarding individual components may vary. Whether or not one would accept blood subfractions—such as immunoglobulins, albumin, and coagulation factor concentrates—also varies between individuals. For this reason, it is vital that physicians engage Jehovah's Witnesses in shared decision making and for patients to make clear what they will or will not accept, even if death is imminent. Documentation of these wishes prior to the acute care setting can be helpful to avoid confusion or undue pressure. Treatment of anemia in this patient population is a challenge in the medical and surgical setting. Blood conservation and the use of adjunctive therapies remain the mainstay of treatment in Jehovah's Witnesses with anemia or preoperatively in anticipation of a fall in hemoglobin. Blood conservation includes minimizing daily phlebotomy for routine labs, utilizing small volume sampling, and careful attention to minimizing blood loss intraoperatively. Supportive measures to prevent or treat anemia include swiftly stopping blood loss, stimulating erythropoiesis (eg, recombinant human erythropoietin, intravenous iron, folic acid and vitamin B₁₂ supplementation), and maintaining blood volume. Substitute blood products such as hemoglobin-based oxygen carriers (HBOC) have been evaluated in

an attempt to provide an alternative to donor-derived red cell products. These products have failed to achieve FDA approval, but may be considered on a compassionate use basis with emergency FDA approval for off-label use.

Blood storage

A recurrent area of controversy is the effect of blood storage time on clinical outcomes of transfusion recipients. Several large randomized studies have shown no difference in outcomes such as mortality based on length of red cell unit storage. While the debate continues, and there may be reasons to choose a particular blood product in an individual patient, the general recommendation is that outcomes do not differ with current blood inventory management protocols.

KEY POINTS

- Life-threatening causes of thrombocytopenia should be considered first in any patient presenting with thrombocytopenia: DITP, HIT, TTP, sepsis and DIC, CAPS, and PTP.
- Prompt treatment should be provided to any patient with a suggested life-threatening cause of thrombocytopenia while awaiting any definitive laboratory diagnosis.
- The diagnosis of TTP should be considered in any patient with thrombocytopenia and microangiopathic hemolytic anemia.
- DIC is characterized by increased thrombin and fibrinolysis. Management is aimed primarily at treating the underlying cause.
- Examination of the peripheral blood film should be part of the investigations for any patient presenting with thrombocytopenia.
- HIT is an uncommon cause of thrombocytopenia in patients admitted to the ICU.
- For most patients, RBC transfusions are not required for nonbleeding critically ill patients with a hemoglobin concentration >7 g/dL or in surgical patients with a hemoglobin concentration of >8 g/dL. The decision to transfuse should be based on an individualized assessment of the patient's clinical status, oxygen delivery needs, and the rate of decline in hemoglobin rather than on a predetermined hemoglobin trigger.

Consultation for hematologic complications of solid organ transplantation

This section offers an approach to the patient with hematologic complications after solid organ transplantation. One of the most common reasons for hematologic con-

sultation in this setting, as illustrated by the clinical case, is single lineage or multilineage cytopenia.

CLINICAL CASE

You are consulted on a 33-year-old woman with thrombocytopenia. She underwent renal transplantation 3 weeks ago for end-stage diabetic nephropathy. Over the past week, she has developed abdominal pain, fever, and increased bruising. Her laboratory studies demonstrate a white blood cell count of 5,000/mL, a hemoglobin of 7.5 g/dL, a platelet count of 52,000/mL, and a serum creatinine of 2.6 mg/dL. There is evidence of nonimmune hemolysis with an elevated lactate dehydrogenase, reticulocytosis, reduced hemoglobin, and negative direct Coombs test. She is taking prednisone and tacrolimus. Blood cultures and viral DNA testing are negative. A peripheral blood smear reveals 5 to 7 schistocytes per high power field. An ADAMTS13 activity returns as 20%. A renal biopsy revealed thrombotic microangiopathy without evidence of graft rejection. You recommend discontinuation of tacrolimus and other nonessential medications.

Drug-related complications

Immunosuppressant and antimicrobial drugs are prevalent causes of cytopenias after solid organ transplantation. Azathioprine is particularly problematic, causing cytopenias in approximately 10% of patients. Because azathioprine and its principal metabolite are cleared predominantly by the kidney, azathioprine-induced marrow toxicity is common following rejection of a renal allograft. Azathioprine toxicity is exacerbated by allopurinol, angiotensin-converting enzyme inhibitors, and trimethoprim/sulfamethoxazole, which frequently are prescribed in the posttransplant setting.

Thrombotic microangiopathy occasionally occurs within the first few weeks after solid organ transplantation in patients treated with calcineurin inhibitors, such as cyclosporine or tacrolimus. In renal transplant patients, this entity may be difficult to distinguish from hyperacute humoral rejection of the allograft without a renal biopsy. Pathologic evidence of thrombotic microangiopathy usually is restricted to the kidneys and often responds to switching, reducing, or withdrawing the offending drug. Solid organ transplantation-related thrombotic microangiopathy, in contrast to idiopathic TTP, is generally not associated with a severe ADAMTS13 deficiency. Although plasma exchange may be attempted in refractory cases, there is little evidence to support its use in this setting.

Clinicians should also be aware that drugs not specific to solid organ transplantation but used in supportive care can cause cytopenias; for example, folate deficiency from

the administration of trimethoprim/sulfamethoxazole, hemolysis from dapsone or trimethoprim/sulfamethoxazole prophylaxis in patients with unrecognized glucose-6-phosphate dehydrogenase deficiency, or drug-induced hemolytic anemia from beta-lactam antibiotics or trimethoprim/sulfamethoxazole.

Infectious complications

While immunocompromised solid organ transplantation recipients are at risk for various opportunistic infections or reactivation of previous infections, those associated with posttransplant cytopenias include parvovirus B19, cytomegalovirus (CMV), human herpesvirus 6 (HHV6), and Epstein-Barr virus (EBV). Strategies for CMV surveillance posttransplant are well established. CMV viremia is associated with leukopenia and thrombocytopenia. Reactivation of latent HHV6 is common after transplant but rarely associated with clinically significant disease. Clinically significant HHV6 reactivation manifests as leukopenia, although other cell lines can be affected. Peripheral blood quantitative polymerase chain reaction is the preferable method of viral detection in immunocompromised patients. The first-line treatment for both CMV and HHV6 viremia includes ganciclovir or foscarnet. Parvovirus B19 infection in immunocompromised patients specifically targets erythroid-lineage cells causing a pure red cell aplasia associated with anemia, marked reticulocytopenia, and erythroid maturation arrest at the pronormoblast stage. Diagnosis can be confirmed by enzyme-linked immunosorbent assay for anti-B19 specific antibodies, quantitative polymerase chain reaction for parvovirus B19 DNA, or by classic findings of giant pronormoblast stage arrest in the bone marrow. Treatment includes reduction of posttransplant immunosuppressive drugs, IVIG, and erythropoietin.

Alloimmune complications

Graft-versus-host disease (GVHD) is a rare and often fatal complication of solid organ transplantation. It is caused by alloreactive passenger T lymphocytes in the transplanted organ. The risk of GVHD is related, in part, to the dose of transplanted lymphocytes. Of all solid organ transplantation, patients receiving small bowel or liver transplantation receive the largest dose of passenger lymphocytes. As such, donors are typically treated with antilymphocyte antibodies or corticosteroids before organ harvesting to minimize the transplantation of donor T lymphocytes. GVHD in solid organ transplantation patients presents similarly to acute GVHD after hematopoietic stem cell transplantation. Fever, rash, and diarrhea 2 to 6 weeks after transplantation

are common initial complaints. Cytopenias, due to GVHD directed against host hematopoietic cells, also may occur and must be distinguished from more common causes of cytopenias in the posttransplant setting, such as drugs and infection. The diagnosis may be confirmed by biopsy of the skin or other affected organs and by peripheral blood chimerism studies, which quantify the proportion of circulating lymphocytes that are of donor and recipient origin. There is no standard therapy for this rare disease. Management generally includes supportive care and immunosuppressive agents. Prognosis is poor and death is typically due to infection from severe marrow aplasia and multiorgan system failure.

Another alloimmune complication of solid organ transplantation is alloimmune hemolysis of host erythrocytes by antibodies produced by donor lymphocytes, also known as passenger lymphocyte syndrome. Like GVHD, passenger lymphocyte syndrome is more common in transplants containing greater numbers of lymphocytes. The syndrome is most common after small bowel transplantation, followed by heart-lung, liver, and kidney transplantation. Passenger lymphocyte syndrome occurs when donor memory B lymphocytes are stimulated after transplant by exposure to recipient or transfused red cell antigens leading to antibodies directed against these antigens. Most cases are due to ABO or Rh(D) incompatibility, but the syndrome also has been reported secondary to incompatibilities with the c, e, JK(a), K, and Fy(a) antigens. Hemolysis is abrupt and occurs several days after transplantation. In addition to classic laboratory markers of hemolysis, the direct Coombs test is positive and serum antibodies against a target recipient red cell antigen are detectable. Most cases can be treated with red cell transfusions of organ donor ABO group compatibility. If hemolysis persists, other treatments include escalation of immunosuppression, intravenous immunoglobulin, rituximab, red cell exchange to remove incompatible host-origin red blood cells, or plasma exchange to remove donor lymphocyte-mediated antibodies. Passenger lymphocyte syndrome is typically self-limited due to the short survival of donor lymphocytes in the circulation. Occasionally, hematologists are asked to comment on the use of apheresis to address human leukocyte antigen (HLA) sensitization or manage antibody-mediated rejection of solid organs. For instance, therapeutic plasma exchange for antibody-mediated rejection is a Class III indication in cardiac and lung transplantation, but carries a Class I indication in ABO-compatible renal transplantation and Class II indication in ABO-incompatible renal transplantation. The reader is referred to the 2016 American Society of Apheresis "Guidelines on therapeutic apheresis"

for practical evidence-based recommendations on apheresis for specific diseases, as well as Chapter 13 for further discussion about appropriate indications for apheresis procedures.

Posttransplantation lymphoproliferative disorders (PTLDs)

Posttransplantation lymphoproliferative disorders (PTLDs) make up a group of predominantly B-cell neoplasms that occur in immunosuppressed individuals following solid organ transplantation. In most cases, B-cell proliferation is induced by EBV infection. PTLD affects ~1% of solid organ recipients and typically occurs within the first year. It is due to impairment of EBV-specific, cytotoxic T-cell function by immunosuppression that allows for expansion of the latent EBV-infected B cells. Principal risk factors for the development of this complication include greater intensity of immunosuppression and receipt of a solid organ from an EBV-seropositive donor by an EBV-seronegative recipient.

Three types of EBV-related PTLD are recognized: benign polyclonal lymphoproliferation, which presents 2 to 8 weeks after initiation of immunosuppression and resembles infectious mononucleosis in presenting symptoms; polyclonal lymphoproliferation with early evidence of malignant transformation; and monoclonal B-cell proliferation with evidence of malignancy by cytogenetics and immunoglobulin gene rearrangements. Patients may present with constitutional symptoms, cytopenias, or lymphadenopathy. Extranodal disease is common. Involved organs include the gastrointestinal tract, lungs, skin, liver, central nervous system, and the allograft itself. The different types of PTLD are diagnosed by a combination of histologic features (eg, underlying architecture), clonality (polyclonal versus monoclonal), immunoglobulin gene rearrangements, and EBV positivity within the context of the clinical scenario.

Treatment depends on the type of PTLD. Benign polyclonal lymphoproliferation and polyclonal lymphoproliferation with early evidence of malignancy typically are managed with a reduction of immunosuppression and antiviral agents. Immunosuppression must be reduced cautiously to reduce the risk of allograft rejection. Patients with monoclonal PTLD rarely respond to reduction of immunosuppression alone. If the PTLD expresses CD20, rituximab may be used alone or in combination with chemotherapy. Single-arm studies suggest response rates of 40% to 70% with rituximab, although randomized controlled trials have not been reported. Radiation therapy may be used for treatment of local disease.

Transfusion support

A hematology consultant may be asked to assist with transfusion management in a patient undergoing solid organ transplantation. Of all solid organ transplantations, RBC, plasma, and platelet transfusion is most commonly required for liver transplantation due to the underlying coagulopathy of liver failure. Heart and heart-lung transplantations frequently require transfusion support, whereas kidney and kidney-pancreas transplantation generally do not require blood product replacement.

Transfusion therapy for solid organ transplantation carries the potential risks of infection, HLA alloimmunization and, rarely, transfusion-associated GVHD. The most frequent transfusion-associated infection complicating solid organ transplantation is CMV. Although CMV viremia usually is due to reactivation in a seropositive immunocompromised recipient, seronegative recipients can acquire CMV through transfusion. To prevent this complication, seronegative recipients should receive transfusions that are CMV-negative or leukocyte reduced.

In the past, transfusions were administered before transplantation as a form of immunomodulation to reduce the risk of solid organ rejection. Randomized studies, however, have shown that modern immunosuppressive agents are more effective at preventing graft rejection than pretransplantation transfusion. Moreover, exposure to allogeneic lymphocytes may induce anti-HLA antibodies, which increase the risk of acute and chronic rejection. To minimize this risk, patients expected to undergo kidney, heart, or lung transplantation should receive blood that is leukocyte reduced. Because of conflicting data, leukocyte reduction is considered optional for patients undergoing liver transplantation. Plasma exchange, IVIG, and rituximab have been used in patients with a positive panel of reactive antibodies or major ABO incompatibility to minimize the risk of hyperacute rejection.

Transfusion-associated GVHD is rare among solid organ transplantation patients, although it is associated with a mortality of 90% or higher as a result of severe pancytopenia. The pathophysiology involves engraftment of donor-derived passenger leukocytes in an immunocompromised host unable to eliminate these passenger leukocytes. Presentation is similar to transplant-associated GVHD and includes skin rash, diarrhea, and liver function abnormalities. While blood product irradiation is believed to reduce the risk of transplant-associated GVHD, this is a rare complication even among immunosuppressed patients and there is no consensus about which patients are most likely to benefit from receiving irradiated blood products.

Posttransplantation erythrocytosis (PTE)

Posttransplantation erythrocytosis (PTE) is defined as an elevated hematocrit exceeding 51% that occurs following renal transplantation and persists for more than 6 months in the absence of leukocytosis, thrombocytosis, or another potential cause of primary or secondary erythrocytosis. PTE affects 8% to 5% of renal transplant recipients; however, the incidence appears to be decreasing. The pathophysiology of PTE is poorly understood, but it likely involves dysregulation of the renin-angiotensin system.

PTE classically presents 8 to 24 months after transplantation. Clinical manifestations include malaise, plethora, headache, and a propensity for both venous and arterial thromboembolism similar to patients with polycythemia vera. First-line therapy in patients with a hemoglobin concentration between 17 and 18.5 g/dL is with an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker. In patients who do not respond to medical therapy and in those with a hemoglobin concentration >18.5 g/dL, therapeutic phlebotomy should be added.

KEY POINTS

- Cytopenias occurring after transplantation of a solid organ may be due to infection, drugs (most commonly azathioprine), GVHD, or PTLD (if marrow involvement is present).
- Major risk factors for PTLD include greater intensity of immunosuppression and receipt of a solid organ from an EBV-seropositive donor by an EBV-seronegative recipient.
- Hemolytic anemia in a solid organ transplantation patient may be due to calcineurin inhibitor-associated thrombotic microangiopathy or to the passenger lymphocyte syndrome.
- Considerations for transfusion support of transplant patients include the risks of HLA alloimmunization; transmission of CMV; and, in rare cases, transfusion-associated GVHD.
- PTE occurs 8 to 24 months after renal transplantation and responds to medical therapy with an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker.

Common outpatient hematology consultations

This section focuses on some of the most common reasons for outpatient hematology consultations. Thrombocytopenia, leukocytosis, and leukopenia are examined in detail. Anemia is covered in other sections.

Mild thrombocytopenia

CLINICAL CASE



A 12-year-old boy with a seizure disorder is referred to you because of thrombocytopenia. His platelet count has gradually decreased from $180 \times 10^9/L$ to $38 \times 10^9/L$ over the past 4 months. His only medication is valproic acid, which started 12 months ago and has led to good seizure control. He is otherwise in good health. He reports no episodes of bleeding, and there are no obvious bruises or petechiae on physical examination. In conjunction with his neurologist, you recommend that he reduce the dose of valproic acid.

Patients with platelet counts in the range of 80×10^9 to $150 \times 10^9/L$ are often referred for outpatient hematology consultation. Determining the onset of the thrombocytopenia is important, which inevitably involves tracing back prior blood counts. New-onset thrombocytopenia may represent a new disease process (primary or secondary ITP, bone marrow infiltration, or myelodysplasia) or a complication of medications or infections. Chronic thrombocytopenia or family history of thrombocytopenia may suggest the possibility of an inherited process, such as a MYH9-related macrothrombocytopenic disorder, which may be discovered during pregnancy when women often have their blood tested for the first time. Other causes of thrombocytopenia include SLE, chronic liver disease typically related to underlying hepatitis C or alcohol with or without hypersplenism, or deficiency of nutrients required for hematopoiesis (vitamin B₁₂, folate, copper) (see video on normal hematopoiesis in online edition). Splenomegaly should be assessed with a physical examination and ultrasound if appropriate. Both HIV and HCV may lead to secondary immune thrombocytopenia.

Mild thrombocytopenia itself is not dangerous, but it may occur as a less severe presentation of a number of disorders that can cause more pronounced thrombocytopenia or have other important health impacts. Thus, the patient should be questioned carefully for signs or symptoms of infection, autoimmune disease, or malignancy, and the physical examination should focus on the assessment of lymphadenopathy, hepatosplenomegaly, skin rashes, stigmata of bleeding, and musculoskeletal abnormalities. An underlying etiology often is not found. Most patients with mild thrombocytopenia (platelet count 100×10^9 to $150 \times 10^9/L$) that is thought to be due to an immune process can be reassured because after 10 years, the risk of developing more severe ITP or another autoimmune disease

is low (approximately 7% and 12%, respectively). DITP was discussed in reference to acutely ill patients but also should be considered in patients with mild thrombocytopenia. Although drugs such as sulfa-containing antibiotics often cause severe thrombocytopenia, others, including the anticonvulsant drug valproic acid, may cause mild thrombocytopenia (and other blood abnormalities) that may be dose dependent. Over-the-counter medications, in particular herbal supplements, should be considered.

As with any hematologic disorder, examination of the peripheral blood film is an essential part of the evaluation. Clumped platelets, as seen with pseudothrombocytopenia; large platelets, as seen with certain inherited macrothrombocytopenic disorders; and small platelets, as seen with Wiskott-Aldrich syndrome, may reveal important clues. Furthermore, abnormal leukocyte or red cell morphology can indicate an underlying disease. Hypersegmented neutrophils and macrocytosis may suggest vitamin B₁₂ deficiency, lymphocytosis may suggest underlying chronic lymphocytic leukemia (CLL), and circulating blasts are consistent with acute leukemia. Dysmorphic red blood cells, hypogranulated neutrophils, or Pelger-Hüet cells may suggest underlying myelodysplastic syndrome (MDS), which may present with isolated thrombocytopenia in up to 10% of patients. Testing for HIV and HCV is warranted in any patient with new onset thrombocytopenia without a clear cause.

There are no guidelines as to when or whether the bone marrow should be examined in patients with mild thrombocytopenia. Although the incidence of a primary bone marrow disorder such as MDS increases with age, recent epidemiologic studies demonstrate that ITP is also common in elderly patients. For patients with typical ITP (ie, isolated thrombocytopenia without other abnormalities on the peripheral blood film or physical examination findings), bone marrow examination generally is not required (American Society of Hematology [ASH] guidelines). A bone marrow examination to rule out bone marrow pathology should be performed if unexplained symptoms arise or other hematologic abnormalities appear. In any case of thrombocytopenia, close follow-up of repeat complete blood counts (CBCs) is warranted to establish the trend and pace of the thrombocytopenia.

Leukocytosis

Patients with unexplained leukocytosis are frequently referred to a hematologist because of concern about an underlying hematologic malignancy; however, most patients with unexplained leukocytosis do not have a hematologic malignancy. A common cause of unexplained leukocytosis is benign neutrophilia in cigarette smokers. Obesity has also been linked with neutrophilia due to underlying inflammation.

In addition to examining the peripheral blood film, a careful history and physical examination are important. Unexplained fever or chills with a new heart murmur may suggest infection, such as bacterial endocarditis. A history of diarrhea may suggest occult infection with *Clostridium difficile*. Lithium or corticosteroid use may indicate a drug-induced leukocytosis. Examinations of the skin, lymph nodes, liver, and spleen size are also important. Patients with exudative pharyngitis, splenomegaly, and lymphocytosis may have infectious mononucleosis. The cell type that is elevated leading to an increase in total leukocyte count also can provide a clue to the underlying diagnosis. A concomitant increase in hemoglobin or platelet count may reflect a myeloproliferative neoplasm. Chronic persistent lymphocytosis with an absolute lymphocyte count of >5,000/ μ L may be the first indication of an underlying chronic lymphocytic leukemia. If the peripheral blood smear shows immature circulating forms or a prominent basophilia, chronic myelogenous leukemia (CML) should be considered. Testing for the BCR-abl translocation is widely available. The myeloproliferative neoplasm/myelodysplastic overlap conditions such as chronic myelomonocytic leukemia (CMML) may have cytopenias with dysplastic findings in conjunction with pronounced monocytosis. Further workup involves a bone marrow biopsy and aspirate. Table 2-4 lists specific causes of leukocytosis according to the predominant cell type that is elevated. Additional laboratory tests such as a bone marrow examination, flow cytometry, and cytogenetics may be required to detect an abnormal malignant clone if malignancy is suspected.

Leukopenia

Leukopenia is defined as a total leukocyte count that is 2 standard deviations below the mean. In evaluating a patient with leukopenia, it is important to check previous CBCs to establish rate of changes. Some racial groups such as Africans, African Americans, and Yemenite Jews may have leukocyte counts that normally fall below the reference range of many laboratories. Notably, these patients have adequate bone marrow neutrophil reserve and are not at increased risk of infection. Leukopenia can be further differentiated by the specific cell type that is affected. Leukopenia results from either decreased marrow production of leukocytes or from decreased circulation of leukocytes due to destruction, margination, or sequestration. Neutropenia can be classified as either congenital or acquired. Congenital forms typically present in childhood with recurrent infections. For example, patients with cyclic neutropenia, due to disorders with neutrophil elastase, typically have a 21-day periodicity associated with

Table 2-4 Hematology consultation for leukocytosis: etiologic considerations according to leukocyte subtype affected

Neutrophilia	Monocytosis	Eosinophilia	Lymphocytosis
Eclampsia	Pregnancy	Allergic rhinitis	Mononucleosis syndrome
Thyrotoxicosis	Tuberculosis	Asthma	Epstein-Barr virus
Hypercortisolism	Syphilis	Tissue-invasive parasite	Cytomegalovirus
Crohn disease	Endocarditis	Bronchopulmonary aspergillosis	Primary HIV
Ulcerative colitis	Sarcoidosis	Coccidioidal infection	Viral illness
Inflammatory/rheumatologic disease	Systemic lupus erythematosus	HIV	Pertussis
Sweet's syndrome	Asplenia	Immunodeficiency	<i>Bartonella henselae</i> (cat scratch disease)
Infection	Corticosteroids	Vasculitides	
Bronchiectasis	Juvenile myelomonocytic leukemia	Drug reaction	Toxoplasmosis
Occult malignancy	Chronic myelomonocytic leukemia (CMML)	Adrenal insufficiency	Babesiosis
Trauma/burn		Occult malignancy	Drug reaction
Severe stress (emotional or physical)		Pulmonary syndromes	Reactive large granular lymphocytosis
Panic		Gastrointestinal syndromes	Chronic lymphocytic leukemia
Asplenia		Hypereosinophilic syndrome	Monoclonal B cell lymphocytosis
Cigarette smoking		Eosinophilic leukemia	Postsplenectomy lymphocytosis
Tuberculosis			
Chronic hepatitis			
Hereditary neutrophilia			
Medications			
Obesity			
Corticosteroids			
β-Agonists			
Lithium			
G-CSF or GM-CSF			
Myeloproliferative neoplasm (CML, PV, ET)			

CML, chronic myelogenous leukemia; PV, polycythemia vera; ET, essential thrombocythemia.

their neutropenia. A list of causes of acquired leukopenias that affect neutrophils, lymphocytes, or both is included in Table 2-5. Congenital neutropenias are reviewed below within the pediatric section.

A careful medication history is important because many drugs, including antibiotics, anti-inflammatory drugs, and anticonvulsants can cause leukopenia. Drug-induced leukopenia can be dose related, as is the case with phenothiazines, or can be immune mediated. Certain medications

are classically associated with agranulocytosis from bone marrow suppression, including clozapine, methimazole, and trimethoprim-sulfamethoxazole, among others. A wide variety of infectious disorders can cause leukopenia, including hepatitis, mononucleosis, HIV, typhoid, and malaria. Cocaine or heroin (contaminated with levamisole) is an increasingly recognized cause of acquired leukopenia in young, otherwise healthy individuals. As with cytopenias of red cell or platelet lineage, autoimmune disor-

Table 2-5 Causes of acquired leukopenia

Infection associated
Postinfectious
Active infection
Sepsis
Viral (HIV, CMV, EBV, hepatitis A, B, C, influenza, parvovirus)
Bacterial (tuberculosis, tularemia, <i>Brucella</i> , typhoid)
Fungal (histoplasmosis)
Rickettsial (Rocky Mountain spotted fever, ehrlichiosis)
Parasitic (malaria, leishmaniasis)
Drug-induced (eg, sulfasalazine, NSAIDs, clozapine, cocaine/levamisole, trimethoprim-sulfamethoxazole, sulfonamides, cephalosporins, dapsone, vancomycin, phenytoin, valproate, deferasirox)
Agranulocytosis
Mild neutropenia
Autoimmune
Primary autoimmune
Secondary autoimmune (systemic lupus erythematosus, rheumatoid arthritis)
Felty syndrome
Malignancy
Acute leukemia
Myelodysplasia
Lymphoproliferative disorder
Large granular lymphocyte leukemia
Plasma cell dyscrasia
Myelophthisic process
Nutritional
Vitamin B ₁₂ or folate deficiency
Copper deficiency
Alcohol
Acute respiratory distress syndrome
Increased neutrophil margination (hemodialysis)
Hypersplenism
Thymoma
Immunodeficiency
Iatrogenic
CMV, cytomegalovirus; EBV, Epstein-Barr virus.

ders, nutritional deficiencies, and hypersplenism can lead to leukopenia. Neutropenia may be seen with rituximab when used either for malignant or nonmalignant disorders.

Patients with leukopenia may be asymptomatic and may not require treatment. Patients who are profoundly leukopenic may complain of fever, mouth sores, or myalgias.

Evaluation of patients with leukopenia includes a careful physical examination, including examination of the mucous membranes and skin. The peripheral blood film should be evaluated for the presence of blasts, which would indicate acute leukemia, or Pelger-Huët cells, which are seen in MDS. Evaluation of the bone marrow with flow cytometry may be helpful to identify a malignant clone. Next-generation sequencing of the peripheral blood or marrow for common mutations seen in MDS has become a helpful adjunct in the evaluation of patients with cytopenias of unclear significance but needs to be considered in the context of morphologic findings before a patient is labeled as having MDS. Ongoing work with next generation sequencing has led to several new terms, including idiopathic cytopenias of undetermined significance, clonal hematopoiesis of indeterminate potential, and clonal cytopenias of undetermined significance. A rheumatologic evaluation, including antinuclear antibody and rheumatoid factor, may indicate a previously undetected collagen vascular disorder or SLE. Splenomegaly in this setting may suggest Felty's syndrome, characterized by the triad of seropositive rheumatoid arthritis, neutropenia, and splenomegaly. Both large granular lymphocytic leukemia and hairy cell leukemia may cause neutropenia and should be considered within the differential diagnosis during evaluation. Treatment of leukopenia depends on the specific etiology. Treatment with colony-stimulating factors should not be used unless there is a definitive diagnosis requiring such an intervention or if severe infection occurs in the setting of neutropenia. Basing initiation of colony-stimulating factors on absolute neutrophil count (ANC) should be used with consideration of the clinical context, with treatment generally given for patients with ANC <500, especially in the setting of an active infection or fever.

Lymphadenopathy

The peak mass of lymphoid tissue occurs in adolescence. In adults, lymph nodes normally are not palpable except for the inguinal region, where small nodes up to 1.5 cm may be felt. Although superficial enlarged nodes can be palpated, deeper nodes require imaging with computed tomography (CT), positron emission tomography, or magnetic resonance imaging (MRI) for detection. Lymph node enlargement can occur in a variety of disorders, including infections, malignancy, and collagen vascular disorders (Table 2-6).

In the primary care setting, more than 98% of enlarged lymph nodes are nonmalignant, whereas 50% of patients referred to a specialist for lymphadenopathy are found to have malignant disease. A thorough exposure and travel history can reveal the source of underlying infections (eg,

Table 2-6 Causes of persistent unexplained lymphadenopathy

Localized	Generalized
Bacterial infection	Mononucleosis syndrome
Fungal infection	Epstein-Barr virus
Tuberculosis	Cytomegalovirus
Other mycobacterial infections	Primary HIV
<i>Bartonella henselae</i> (cat scratch disease)	Chronic HIV
Sarcoidosis	Other viral infections
Langerhans cell histiocytosis	Leptospirosis
Inflammatory pseudotumor	Tularemia
Progressive transformation of germinal centers	Miliary tuberculosis
Malignancy (eg, NHL, HD, CLL, metastatic carcinoma)	Brucellosis
	Lyme disease
	Secondary syphilis
	Toxoplasmosis
	Histoplasmosis, coccidiomycosis, cryptococcosis
	Systemic lupus erythematosus
	Rheumatoid arthritis
	Still's disease
	Rosai-Dorfman disease
	Sarcoidosis
	Langerhans cell histiocytosis
	Phenytoin
	Drug-induced serum sickness
	Castleman disease
	Kikuchi disease
	Kawasaki disease
	Angioimmunoblastic lymphadenopathy
	Atypical lymphoproliferative process (eg, Castleman disease)
	Autoimmune lymphoproliferative syndrome
	Hemophagocytic lymphohistiocytosis
	Malignancy (eg, indolent NHL, HD, CLL, metastatic carcinoma)

CLL, chronic lymphocytic leukemia; HD, Hodgkin disease; NHL, non-Hodgkin lymphoma.

cat scratch and *Bartonella henselae*, undercooked meat and toxoplasmosis, tick bite and Lyme disease, high risk behavior and HIV). Constitutional symptoms such as fevers, night sweats, and weight loss may suggest infection or malignancy. Localizing signs and symptoms of an infection should be elicited. Review of the medication list may reveal a drug (eg, phenytoin) that is associated with lymphadenopathy. On physical examination, large size, hard texture, fixed mobility, asymmetry, and the lack of pain are features suggestive of malignancy. The patient should be

evaluated for splenomegaly as well. Additional laboratory investigations for patients with lymphadenopathy might include antinuclear antibody, rapid plasma reagent, testing for tuberculosis, monospot, HIV, CBC, and review of the peripheral blood smear. Patients with localized lymphadenopathy can be observed for a few weeks provided no other concerning features on history or physical exam exist. Tissue biopsy is required to determine the precise etiology of lymphadenopathy. If a hematologic malignancy is suspected, an excisional lymph node biopsy should be

performed to preserve the tissue architecture. Fine-needle aspirations often provide a sample of tissue that is inadequate for making the diagnosis of lymphoma. Lymph node biopsy specimens should be sent for flow cytometry, cytogenetics, appropriate molecular genetic testing, and immunohistochemistry.

Castleman disease (angiofollicular lymph node hyperplasia) is a lymphoproliferative disorder characterized by polyclonal expansion of plasma cells and B and T lymphocytes and increased interleukin 6 levels leading to localized or systemic lymphadenopathy. The disease is categorized as unicentric, involving one lymph node region (typically in the chest), or as multicentric, with generalized lymphadenopathy. Unicentric Castleman disease can be classified pathologically into hyaline vascular variant, plasmacytoid variant, and human herpesvirus 8 (HHV8)-positive Castleman disease. HHV8 encodes a viral interleukin 6-protein and has been implicated especially in patients with HIV. Unicentric disease of the hyaline vascular variant is typically treated with radiation therapy or local resection. Mixed histology, plasmacytoid variants, and multicentric disease can present with B-symptoms, organomegaly, and cytopenias. These aggressive subtypes may progress to lymphoma and require lymphoma-type treatment. Antiviral agents, such as ganciclovir, have been investigated in HIV-positive patients with HHV8-positive disease.

Splenomegaly

The normal adult spleen measures up to 13 cm in largest diameter, weighs approximately 150 g, and is not palpable. Splenic enlargement frequently is not appreciated on physical examination unless the spleen size is increased by 40%. Spleen size typically is quantified by measuring splenic extension below the costal margin in centimeters. Splenic enlargement is best appreciated on physical examination when there is percussive dullness in Traube's semilunar triangle bordered by the left sternal border, the costal margin, and lower border of the ninth rib. Ultrasonography can accurately determine the size of the spleen, and CT or MRI can be useful in assessing architectural changes due to infarction, infection, infiltration or tumor. Doppler should be obtained along with ultrasound to detect any changes in splenic and portal blood flow to account for splenomegaly.

Splenomegaly occurs in patients with cirrhosis, heart failure, or splenic vein thrombosis when increased portal pressure causes venous engorgement and disruption of the normal splenic architecture. Other causes are splenic infarction, hematologic malignancy such as lymphoma, primary myelofibrosis, infection, and infiltrative disorders such as

Gaucher disease. Splenomegaly can be seen in conditions of ongoing hemolysis such as hereditary spherocytosis or when there is extramedullary hematopoiesis as is seen in severe thalassemia. Solid tumor malignancies rarely metastasize to the spleen. Normally, about one-third of circulating platelets are sequestered in the spleen, where they are in equilibrium with circulating platelets; thus, splenomegaly can cause cytopenias (termed *hypersplenism*) because of increased splenic sequestration. In these instances, the apparent thrombocytopenia rarely is associated with clinical bleeding or requirements for platelet transfusion since the total platelet mass and overall platelet survival remain relatively normal. However, in chronic liver disease where patients have multiple hemostatic issues (eg, true thrombocytopenia from decreased thrombopoietin production, decreased production coagulation factors and fibrinogen, hyperfibrinolysis, bone marrow suppression from HCV, and anatomical variceal bleeding) or require medical/surgical procedures, thrombocytopenia due to hypersplenism may contribute to the risk of bleeding. The initial evaluation of a patient with splenomegaly includes a detailed history to determine any of the above underlying causes and a thorough physical exam focusing on lymph nodes, spleen, and liver. Laboratory tests may include a CBC, peripheral smear, liver function tests, urinalysis, HIV test, and chest x-ray. Imaging to evaluate malignancy or liver disease should be considered if the above initial testing is unrevealing. Biopsy of affected tissues (eg, lymph nodes, liver, or bone marrow) may be pursued if the cause of splenomegaly is not obvious, as splenic biopsy/aspiration is typically not performed due to significant risks. Peripheral blood flow cytometry may show evidence of an underlying lymphoproliferative disorder, such as hairy cell leukemia or marginal zone lymphoma.

Diagnostic and therapeutic splenectomy may be indicated for patients with massive splenomegaly causing pain from infarction or recalcitrant cytopenias. Splenectomy may be indicated for patients with hereditary spherocytosis, ITP, or warm antibody-mediated hemolytic anemia. Because of the risk of rapidly progressive septicemia from encapsulated organisms, in patients with surgical, functional, or congenital asplenia, these patients should be vaccinated for *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*. Prophylactic antibiotics are recommended for asplenic children under the age of 5, should be considered for 1 to 2 years postsplenectomy in older children and adults, and should be continued as lifelong prophylaxis for any asplenic individual who has a history of postsplenectomy sepsis. It is critically important that despite these prophylactic measures, asplenic individuals who develop a fever should be treated promptly with therapeutic

antibiotics. Splenectomy may be associated with a long-term increased risk of vascular complications and pulmonary hypertension, particularly when performed for diseases with increased RBC turnover. In these cases, aggressive VTE prophylaxis should be administered to prevent thromboembolic complications postoperatively. On the peripheral blood film, Howell-Jolly bodies (nuclear remnants within RBCs) most often indicate the absence of the spleen from splenectomy or splenic hypofunction, as in sickle cell disease. After splenectomy, patients are often noted to have chronic leukocytosis and thrombocytosis.

Splenic artery embolization has been described in several case reports as effective for managing the hematologic sequelae of portal hypertension such as thrombocytopenia. While those unable to undergo splenectomy may benefit from this approach, larger studies are needed to define efficacy and the ideal patient population prior to increasing use.

Gaucher disease is a lysosomal storage disorder caused by deficiency of glucocerebrosidase. Splenomegaly with concomitant thrombocytopenia and hepatomegaly are the most common clinical manifestations, while some forms demonstrate developmental delay or other neurologic disease. Diagnosis is by demonstrating decreased leukocyte glucocerebrosidase activity or by mutational analysis. Treatment with enzyme replacement therapy improves symptoms and quality of life.

KEY POINTS

- Most patients with stable, mild thrombocytopenia (platelets $100 \times 10^9/L$ to $150 \times 10^9/L$) do not develop worsening thrombocytopenia or other autoimmune diseases.
- Thrombocytopenia caused by medications may be immune mediated or dose dependent.
- Hard, fixed, nontender, and enlarged lymph nodes may be features suggestive of malignancy.
- An excisional lymph node biopsy is better than a fine needle aspiration for making a tissue diagnosis of lymphoma.
- Patients requiring splenectomy should be vaccinated against encapsulated bacteria to reduce the risk of overwhelming postsplenectomy infection.

Hematology consultations in pediatric patients

Pediatric consultation requires evaluation based on knowledge of developmental hematology and distinct etiologies that are not present in other patient populations. These key issues are discussed in this section.

Anemia

Following is an overview of anemia in the pediatric population. For additional information on individual conditions, refer to Chapters 5 to 8.

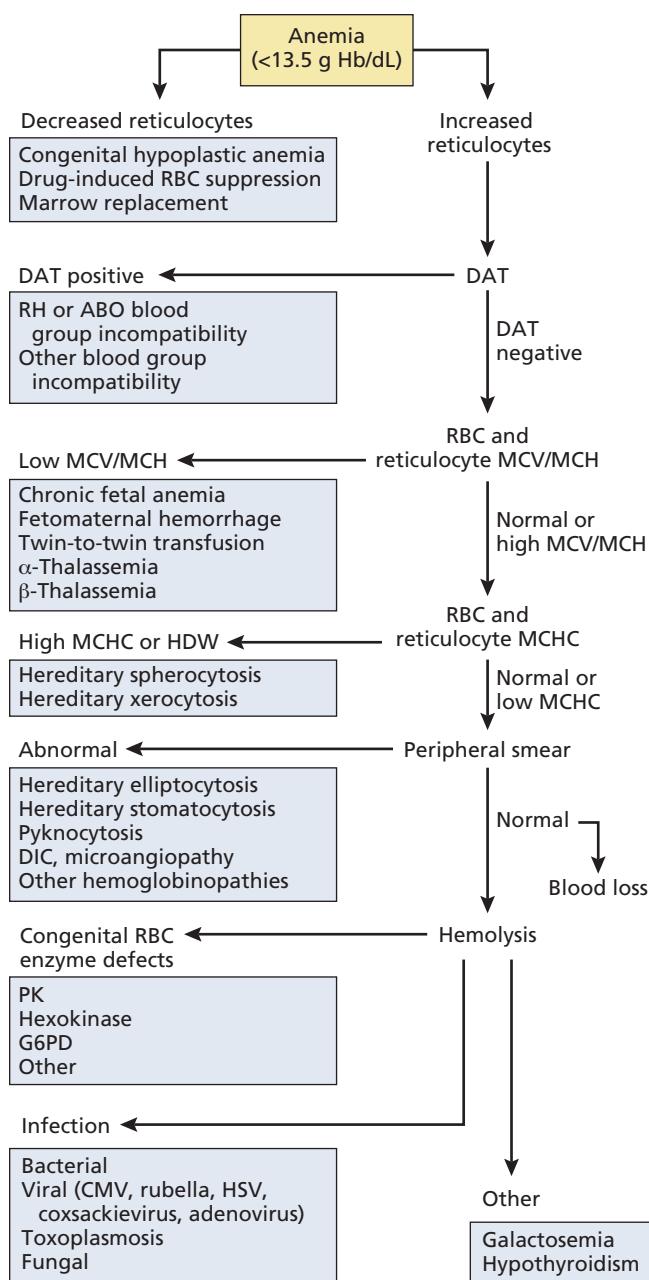
Newborns

Figure 2-3 illustrates the diagnostic approach to anemia in the newborn. At birth, infants are relatively polycythemic and macrocytic, reflecting fetal RBC production in the hypoxic intrauterine environment. Mean hemoglobin and hematocrit on day 1 of life for a term newborn are elevated (Table 2-7) and, therefore, a hematocrit that would be considered normal during childhood represents anemia in the newborn. Shortly after birth, erythropoietin declines, and by day 7, the reticulocyte count is 0.5%, resulting in a physiologic nadir hemoglobin concentration ($10.7 \pm 0.9 \text{ g/dL}$) at approximately 7 to 9 weeks of age. This nadir can occur earlier and be more pronounced in premature infants.

Careful assessment of the obstetrical and birth history, along with review of the family history for jaundice, anemia, splenectomy, or cholecystectomy, can assist in identifying the cause of anemia in the newborn. Physical examination should focus on findings such as jaundice, vital signs, and possible sources of internal blood loss. A review of the CBC, red cell indices, reticulocyte count, and peripheral blood film can narrow the broad differential. Additional laboratory testing should be guided by the presence or absence of findings.

Neonatal anemia can be classified as caused by blood loss, increased RBC destruction, or decreased RBC production. Blood loss can result from placenta previa or rupture of an abnormal umbilical cord. Acute or chronic fetal-maternal hemorrhage and internal hemorrhage in the infant must also be excluded. Depending on the extent of blood loss, the infant may have signs and symptoms of circulatory shock. In the setting of chronic blood loss, the infant may be compensated but exhibit pallor and in severe cases congestive heart failure. Fetal-maternal hemorrhage can be confirmed, and the quantity of blood loss estimated, by the Kleihauer-Betke test on maternal blood. An uncommon source of blood loss is the twin-twin transfusion syndrome, defined as a 5 g/dL or more difference in hemoglobin concentration between twins. Hemorrhage can be acute or chronic, with variable presentations and the potential for polycythemia in the recipient twin.

Hemolytic anemia in the newborn may be classified as either intrinsic or extrinsic. Extrinsic causes include alloimmune-mediated destruction, infection, DIC, and severe acidosis. Intrinsic causes include enzyme deficiencies, membrane defects, and hemoglobinopathies. The infant

**Figure 2-3 Diagnostic approach to anemia in the newborn.**

From Brugnara C, Platt OS, in Nathan DG et al, eds., *Nathan and Oski's Hematology of Infancy and Childhood*, 6th ed. (Philadelphia, PA: WB Saunders; 2003:19–55). CMV, cytomegalovirus; DAT, direct antiglobulin test; DIC, disseminated intravascular coagulation; G6PD, glucose-6-phosphate dehydrogenase; HDW, hemoglobin distribution width; HSV, herpes simplex virus; MCHC, mean corpuscular hemoglobin concentration; MCV/MCH, mean corpuscular volume/mean corpuscular hemoglobin; PK, pyruvate kinase.

usually will demonstrate a normocytic anemia with an increase in the reticulocyte count. Immune causes of anemia are becoming increasingly rare in developed countries, given the widespread use of prenatal screening and Rh-immune globulin administration to Rh-negative women. However, Rh-isoimmunization is still the most common cause of neonatal alloimmune hemolytic anemia worldwide. Immune hemolysis due to ABO incompatibility, currently the most common cause of hemolytic disease of the newborn in countries with a high human development index, is most likely in the setting of an A infant and O mother, given that maternal isoantibodies titers are usually higher for A than for B and that expression of the A antigen on neonatal RBCs is usually higher than expression of the B antigen. If suspected, laboratory testing includes maternal and infant red cell and Rh typing along with a direct antiglobulin test (DAT) in the infant. The peripheral blood film shows a variable amount of spherocytes depending on the degree of hemolysis. A negative DAT does not exclude the diagnosis of incompatibility because A antigen density may be too low to cause cross-linking in the test. Other than Rh and ABO, anti-Kell antibodies may produce severe disease in up to 40% to 50% of affected fetuses. Common intrinsic red cell etiologies include hereditary spherocytosis and glucose-6-phosphate dehydrogenase (G6PD) deficiency, which will be discussed in the following paragraphs.

Impaired RBC production is less common, but it should be considered in any infant with isolated anemia and inappropriately low reticulocyte count. Causes include congenital infections, particularly toxoplasmosis, rubella, CMV, and herpes simplex (TORCH infections); drug-induced suppression; and, rarely, Diamond-Blackfan anemia (DBA), or other disorder of red cell production.

Management requires evaluation of the possible cause, severity, and hemodynamic status of the infant. Stable infants with mild anemia may be followed with close observation. Infants with more severe anemia can be managed with packed RBCs. Slow transfusions or exchange transfusion should be considered in infants with severe anemia and cardiovascular compromise. Specific thresholds for transfusions vary among centers and have been studied mostly in premature and low-birth-weight infants. Studies comparing restrictive (low) versus liberal (high) hemoglobin thresholds showed only minimal differences in frequency of transfusions and hemoglobin levels and did not have any impact on combined outcomes of mortality or major morbidity. In a Cochrane review of transfusion thresholds in children without respiratory support, hematocrits of 30%, 25%, and 23% were suggested as thresholds at 1, 2, and ≥3 weeks, respectively. In cases of significant

Table 2-7 Normal hematologic values for newborns

Red blood cell parameter	Term newborn day 1 ± SD*	
Hb (g/dL)	19.0 ± 2.2	
Hct (%)	61 ± 7.4	
MCV (fL)	119 ± 9.4	
Reticulocytes (%)	3.2 ± 1.4	
Coagulation/inhibitor parameter	Healthy term newborn cord blood†	Healthy preterm (30–38 weeks) cord blood†
PT (seconds)	16.7 (12–23.5)	22.6 (16–30)
INR	1.7 (0.9–2.7)	3.0 (1.5–5.0)
aPTT (seconds)	44.3 (35–52)	104.8 (76–128)
Fibrinogen (von Clauss; g/L)	1.68 (0.95–2.45)	1.35 (1.25–1.65)
Factor II activity (%)	43.5 (27–64)	27.9 (15–50)
Factor V activity (%)	89.9 (50–140)	48.9 (23–70)
Factor VII activity (%)	52.5 (28–78)	45.9 (31–62)
Factor VIII activity (%)	94.3 (38–150)	50 (27–78)
Factor IX activity (%)	31.8 (15–50)	12.3 (5–24)
Factor X activity (%)	39.6 (21–65)	28 (16–36)
Factor XI activity (%)	37.2 (13–62)	14.8 (6–26)
Factor XII activity (%)	69.8 (25–105)	25.8 (11–50)
Antithrombin III activity (%)	59.4 (42–80)	37.1 (24–55)
Protein C activity (%)	28.2 (14–42)	14.1 (8–18)
Protein C antigen (%)	32.5 (21–47)	15.9 (8–30)
Total protein S (%)	38.5 (22–55)	21.0 (15–30)
Free protein S (%)	49.3 (33–67)	27.1 (18–40)

From Reverdieu-Pochat P, Delahousse B, Body G, et al. *Blood*. 1996;88:900–906.

aPTT, activated partial thromboplastin time; Hb, hemoglobin; Hct, hematocrit; INR, international normalized ratio;

MCV, mean corpuscular volume; PT, prothrombin time.

* Adapted from Mattoth Y, Zaizov R, Varsano I. *Acta Paediatr Scand*. 1971;60:317–323.

†Values are means, followed by lower and upper boundaries, including 95% of population.

anemia from blood loss, supplemental oral iron should be provided for the first several months of life. Additionally, premature infants will have lower total-body iron stores than normal and should be supplemented with oral iron.

Children

Asymptomatic anemia often is discovered incidentally at approximately 12 to 15 months of age when children undergo a screening hemoglobin. This isolated value, however, does not identify the cause of anemia, and follow-up studies, including a CBC and reticulocyte count, are recommended. This section provides an overview, and details of specific diagnoses are discussed in the specific chapters on anemia. Classification of anemia based on red blood

cell size (mean corpuscular volume [MCV]) and reticulocyte count provides a practical approach to the child with anemia.

Microcytic anemia most often is due to iron deficiency anemia (IDA) or thalassemia. IDA is commonly diagnosed around 1 to 2 years of age. Maternal iron stores become exhausted after 6 months, and thereafter, the child must take in enough dietary iron to maintain hematopoiesis. Although the iron from breast milk is more bioavailable than that from cow's milk, it is generally inadequate as a sole source of iron beyond 4 to 6 months of life. In addition, at 1 year of life, children typically switch to iron-poor cow's milk, have inadequate intake of iron-containing foods, and develop gastrointestinal irritation with poor absorption and occult blood loss secondary to cow milk pro-

teins. A careful diet history usually provides evidence that the child has IDA even without laboratory studies. Older children or children without an obvious dietary explanation should be evaluated for blood loss. Common sites include gastrointestinal, such as inflammatory bowel disease or celiac disease, or menstrual loss in girls. Less common are anatomic abnormalities such as a Meckel diverticulum or double uterus, pulmonary hemosiderosis, or Wegener granulomatosis. Other causes of microcytic anemia include lead poisoning and sideroblastic anemia. Direct and repetitive questioning and specific testing may be required to elicit the cause.

A full discussion of the laboratory evaluation for IDA can be found in Chapter 6; in children, however, additional studies are often not necessary if history, CBC, and red cell indices are highly suggestive. The best confirmatory test for IDA is response to a therapeutic trial of iron. Within 2 weeks of appropriate iron replacement (4 to 6 mg/kg/d of elemental iron), reticulocytosis and improvement of hemoglobin should be observed. The most common reasons children fail iron therapy include nonadherence, improper dosing, and a diagnosis other than IDA. If there is no response to an adequate trial of iron and parents report adherence, this treatment should be stopped and alternative causes, including blood loss and malabsorption, should be sought. Recent advances in the safety of IV iron makes this an option for children who require ongoing iron replacement, have poor iron absorption, or do not tolerate oral iron.

The most common alternative diagnosis is thalassemia, particularly in children of African American, Mediterranean, or Asian backgrounds. The gene deletions and corresponding nomenclature for thalassemia are discussed in Chapter 7. Review of the newborn screening result is helpful in determining α -thalassemia; however, after hemoglobin switching, 1- or 2-gene α -thalassemia will not be evident on hemoglobin electrophoresis. β -thalassemia trait or intermedia may not be detected on newborn screening; however, β -thalassemia major will have a hemoglobin F-only pattern. Later hemoglobin electrophoresis will reveal increased hemoglobin A_2 . It is important to make the correct diagnosis so that children with thalassemia are not inappropriately treated with iron and genetic counseling can be provided. Another common and important—and often unrecognized—cause of microcytic or normocytic anemia is anemia of chronic inflammation (discussed in Chapter 6).

Normocytic anemia poses a greater diagnostic dilemma for the consulting physician. Common causes include: (i) early or rapid blood loss, (ii) hemolytic anemia, (iii) anemia of inflammation, and (iv) transient erythroblastopenia of

childhood (TEC). Information obtained from the history and physical may assist in the diagnosis, including onset of symptoms, personal or neonatal history of jaundice or blood loss, or family history suggestive of hemolytic anemia (jaundice, splenectomy, transfusions, and cholecystectomy). Physical examination may reveal splenomegaly and jaundice in the setting of hemolytic anemia. Vital signs can provide a clue to the duration of anemia based on hemodynamic compensation. Finally, inclusion of the reticulocyte count will help differentiate children with hemolytic anemia and a review of the peripheral blood film often provides the diagnosis.

Extrinsic causes of hemolytic anemia include immune-mediated destruction, microangiopathic destruction (DIC, TTP, and HUS covered in the previous section), and medications. Primary autoimmune hemolytic anemia can be caused by either IgG (warm-reactive) or IgM (cold-reactive) antibodies and presents with the acute onset of uncompensated anemia. While management is similar to that for adults (see Chapter 8), unlike adults, children with autoimmune hemolytic anemia have a good prognosis, with approximately 77% having an acute self-limited condition. Intrinsic causes of hemolytic anemia can be further classified by cause, including: (i) enzyme deficiencies (G6PD), (ii) membrane defects (such as hereditary spherocytosis), or (iii) hemoglobinopathies (sickle cell disease). Each of these is reviewed in detail in Chapters 7 and 8. In all cases, the child usually will demonstrate a normocytic anemia with an increase in the reticulocyte count; however, macrocytosis can occur in the setting of a robust reticulocyte response.

Special mention should be given to TEC, a normocytic anemia with reticulocytopenia resulting from brief disruption of normal erythropoiesis in children. Spontaneous recovery occurs with subsequent brisk reticulocyte response that often mimics acute hemolytic anemia. TEC should be suspected in an otherwise healthy child with acute onset of anemia and no abnormalities on physical examination or peripheral blood film.

Macrocytosis in childhood should always cause concern, and a bone marrow evaluation should be undertaken to look for causes of marrow failure. In early childhood, the diagnosis of DBA, a congenital pure red cell aplasia, should be considered. A quarter of patients with DBA have macrocytic anemia at birth, and 25% of children will have at least one congenital anomaly, including head or face, palate, limb, or kidney abnormalities. Patients have elevated red cell adenosine deaminase activity and fetal hemoglobin levels. Bone marrow evaluation shows a normocellular bone marrow with striking paucity of erythroid precursors. Approximately 25% of DBA patients have heterozygous

mutations in the ribosomal protein S19 (*RPS19*) gene, and mutations in at least 5 other ribosomal protein genes now have been identified. Treatment modalities include corticosteroids, chronic transfusions, and bone marrow transplant. Other causes of bone marrow failure should also be considered (eg, Fanconi anemia) and are covered in Chapters 16 and 19.

Neutropenia

Newborns

Neutropenia in the newborn is relatively common, secondary to the limited neonatal marrow capacity. Therefore, consumption in response to sepsis, respiratory distress, or other acute processes may exceed production. Neutropenia also may be seen in association with in utero stress due to pregnancy-induced hypertension. In both cases, the neutropenia is transient and resolves with resolution of the underlying illness or, in the case of pregnancy-induced hypertension, within 3 to 5 days of delivery.

Neonatal alloimmune neutropenia (NAIN) results from the transplacental passage of maternal antibodies that react with paternal antigens on the infant's neutrophils. The diagnosis of NAIN generally can be made by confirming antigenic differences between maternal and paternal neutrophils, most commonly the NA1 and NA2 alleles, and by demonstrating maternal antibodies that bind to paternal neutrophils. Neutropenia can be profound, with the potential for sepsis, omphalitis, cellulitis, and other serious infections. Granulocyte colony-stimulating factor (G-CSF; 5 mg/kg/ dose) is indicated in severe cases. The condition typically resolves in weeks to months once maternal antibodies are no longer present.

NAIN must be differentiated from relatively rare inherited causes of neutropenia that will be discussed in the following section. In these conditions, the neutrophil count remains severely depressed and children are at risk for ongoing infections.

Children

Neutropenia in children is defined as an ANC $<1.5 \times 10^9/L$. Outside of the neonatal period, it can be classified as either acquired or inherited. Acquired causes include infection, drug-induced neutropenia, and autoimmune or chronic benign neutropenia. Autoimmune neutropenia (AIN) and chronic benign neutropenia of childhood likely represent a spectrum of disorders caused by immune destruction of neutrophils. The condition usually presents in children less than 3 years of age and for the most part is not associated with serious infections. In the majority of children, anti-neutrophil antibodies can be detected; however, due to the

poor sensitivity of antibody testing, a negative result does not exclude the diagnosis. Yield may be increased by repeating the test if clinical suspicion is high. Management is directed at treating infections with antibiotics, and G-CSF should be reserved for those patients with severe or recurrent infections associated with a low absolute neutrophil count. Prognosis is excellent, with spontaneous recovery occurring in almost all patients within 2 years of diagnosis.

A common cause of neutropenia is differences in ethnic neutrophil norms. Certain ethnic populations, particularly African Americans, may have lower normal limits. Usually these children have mild neutropenia (absolute neutrophils between 1.0 and $1.5 \times 10^9/L$), no history of infection or other concerning features on physical examination, and the value will be relatively stable over time. Reassurance is all that is necessary in this setting. Inherited causes of neutropenia represent a rare group of disorders, including severe congenital neutropenia (SCN), Shwachman-Diamond syndrome, and cyclic neutropenia. SCN, an autosomal recessive premalignant condition caused by mutations in the *ELA2* gene, is often diagnosed on the first day of life, and patients have persistent neutropenia associated with frequent episodes of infections. Bone marrow evaluation shows myeloid maturation arrest at the myelocyte stage. Shwachman-Diamond syndrome includes neutropenia, pancreatic exocrine insufficiency, metaphyseal chondrodysplasia, and short stature. Lastly, cyclic neutropenia is an autosomal dominant condition in which patients experience severe neutropenia and associated infections approximately every 21 days. Bone marrow evaluation will look similar to SCN during the nadir, and it may be difficult to distinguish from other causes of neutropenia at first. Careful monitoring with frequent blood counts 1 to 2 times a week for 6 to 8 weeks can help confirm the diagnosis. In all cases, treatment with G-CSF is the standard of care. Less clear is the role of bone marrow transplant for those conditions that are considered premalignant.

Thrombocytopenia

Newborns

Thrombocytopenia in a neonate is defined as a platelet count $<150 \times 10^9/L$ with severe thrombocytopenia generally being reserved for infants with a platelet count $<50 \times 10^9/L$. As with neutropenia, limited capacity of the neonatal marrow to increase platelet production in the face of rapid consumption can result in thrombocytopenia in the sick newborn with estimates of almost 25% of neonates in the neonatal ICU experiencing thrombocytopenia, which can be classified as early or late. Within the first 72 hours, thrombocytopenia is usually the result of

antenatal or perinatal events such as perinatal asphyxia, intrauterine growth restriction, maternal hypertension, intrauterine infection, and intrauterine viral infections. It may also result from immune destruction. After 72 hours, thrombocytopenia is more likely due to postnatal events, including necrotizing enterocolitis and late onset sepsis.

In an otherwise well infant, immune thrombocytopenia should be investigated. Knowledge of maternal medical history and platelet count is critical because management varies depending on suspicion of alloimmune versus autoimmune thrombocytopenia. Autoimmune thrombocytopenia, either primary or secondary, presents early in infancy due to transplacental passage of maternal platelet-reactive IgG (secondary to either ITP or SLE), which binds to common antigens on the infant's platelets. The mother may or may not have thrombocytopenia, as even a remote history of resolved ITP in the mother can lead to transfer of antibodies to the infant. The risk of bleeding is low, and infants often can be managed with observation alone without need for treatment. If the infant does require treatment, then IVIG can be given. Primary ITP in a child generally does not occur earlier than 6 months of age.

NAIT should be suspected in an infant born with severe thrombocytopenia, especially if maternal history is negative and maternal platelet count is normal. NAIT results from the transplacental passage of maternal antibodies that are reactive against paternal-derived antigens expressed on the infant's platelets. This condition is analogous to Rh disease, in that the mother lacks the antigen and the infant inherits the antigen from the father. Unlike Rh disease, however, first pregnancies may be affected by NAIT. The majority of NAIT cases (80%) arise as a result of a maternal antibody against HPA-1a. Other antigens, including HPA-5b and HPA-3b, are less common. Thrombocytopenia caused by NAIT is associated with a high risk of intracranial hemorrhage (10% to 20%); therefore, NAIT should be suspected in any healthy infant with severe thrombocytopenia and prompt management should be initiated. All infants with NAIT should be investigated for intracranial hemorrhage with either ultrasound or CT scan. Treatment is recommended for a platelet count $<30 \times 10^9/L$ or $<100 \times 10^9/L$ in infants with severe hemorrhage. Optimal treatment includes transfusion of HPA-compatible platelets, which can be collected and washed from the mother or from an antigen-negative donor. Random donor platelets should be given if antigen-negative platelets are unavailable since platelet count increments have been documented with this approach. IVIG (1.0 g/kg/d for 1 to 3 days depending on response) and methylprednisolone also may decrease the rate of platelet destruction and can be used as adjunctive therapy. Regardless of treatment, NAIT

usually resolves within 2 to 4 weeks. Specific testing for NAIT, including platelet antigen typing and antibody identification, can confirm the diagnosis; however, treatment should be instituted even if results of testing are unavailable. NAIT testing is important because of the implications for subsequent pregnancies where the risk of severe thrombocytopenia is higher and can occur as early as the second trimester. Prenatal management, risk stratification, and counseling of female family members is recommended and should be undertaken in conjunction with a high-risk obstetrician.

Outside of NAIT, which carries a high risk for bleeding, the role of prophylactic platelet transfusions and desired thresholds for transfusion to prevent bleeding remain unclear. The majority of studies that have been conducted in this area have assessed platelet count, not bleeding events, as the primary outcome, making conclusions about true clinical utility difficult to draw. In one randomized trial, there was no reported increased risk for intraventricular or periventricular hemorrhage in neonates with moderate thrombocytopenia, defined as a platelet count of 50 to $150 \times 10^9/L$. Further studies are needed in this area to determine best practice.

Children

Causes of childhood thrombocytopenia generally can be classified as either due to platelet destruction or impaired platelet production. The most common cause of isolated thrombocytopenia is ITP, which can be either primary or secondary in children. Specific features of ITP in children are outlined here.

ITP is a diagnosis of exclusion based on findings of isolated thrombocytopenia in an otherwise healthy child without abnormalities on physical examination or laboratory studies, including detailed evaluation of the peripheral blood film. A bone marrow examination is not considered necessary for the diagnosis of ITP.

Treatment of the child with ITP remains controversial. Published guidelines by ASH recommend that children with no or mild bleeding do not require treatment regardless of the platelet count. This was based on evidence that the majority of children will experience spontaneous recovery of their platelet count, treatment is unlikely to alter the course of the disease, and severe hemorrhage is a rare event even in children with severe thrombocytopenia. In addition to bleeding symptoms, physicians need to consider quality of life, access to care, and child behavior when determining therapy. When drug therapy is indicated, prospective randomized studies have demonstrated that IVIG and anti-D (in Rh-positive patients) lead to the most rapid increase in platelet count. Although anti-D is easier to

administer, it has been associated with fatal intravascular hemolysis and DIC, which led to a black box warning by the US Food and Drug Administration. Short courses of corticosteroids are effective and much less costly, but they take longer to increase the platelet count. Long courses of corticosteroids are not recommended in children.

In contrast to adult ITP, the majority of children will have an acute course with 75% of patients achieving a complete remission by 6 months from presentation. For patients with persistent or chronic disease, treatment options include intermittent use of medications, splenectomy, or modalities such as rituximab, high-dose dexamethasone, and thrombopoietin receptor agonists. The benefit of splenectomy is a high rate of durable remission, which occurs in approximately 75% of patients; however, this must be weighed against the risks associated with surgery, a life-long risk of sepsis, and possible risk of thrombosis. Rituximab and high-dose dexamethasone have been used in children with chronic ITP to avoid or delay splenectomy, with complete remission rates of approximately 20% to 30%; however, remission duration is generally shorter than with splenectomy. Thrombopoietin receptor agonists are approved for the treatment of ITP in adults, and studies in children have shown them to be safe and efficacious.

Additional causes of thrombocytopenia in children due to destruction include microangiopathic conditions and HIT (rare in children), both discussed in the adult section. Autoimmune lymphoproliferative syndrome results from impaired *fas* ligand-mediated apoptosis. Patients experience recurrent lymphadenopathy, organomegaly, and immune cytopenias. Kasabach-Merritt phenomenon is characterized by thrombocytopenia and microangiopathic anemia associated with the vascular tumors Kaposiform hemangioendothelioma and tufted angioma, and usually presents in early childhood. Patients can develop a severe life-threatening consumptive coagulopathy, and many treatment modalities have been described, including corticosteroids, vincristine and, recently, sirolimus.

Causes of decreased platelet production include aplastic anemia, MDS, bone marrow infiltration, and inherited thrombocytopenias. The inherited thrombocytopenias represent a diverse group of disorders (see Chapter 11). In all cases, a detailed review of the family history, physical examination looking for additional anomalies, and evaluation of platelet and white cell morphology on the peripheral blood film provide important diagnostic clues. Microthrombocytopenia in males should raise the concern for Wiskott-Aldrich syndrome or X-linked thrombocytopenia, caused by a mutation in the *WAS* gene. Wiskott-Aldrich syndrome, unlike X-linked thrombocytopenia, is associated with immune deficiency, and patients require

early identification and management in coordination with an immunologist. Several conditions are characterized by macrothrombocytopenia: *MYH9*-related disease (autosomal dominant), Bernard-Soulier syndrome (autosomal recessive), *GATA1* mutations (X-linked recessive), and gray platelet syndrome (variable inheritance). Normocytic thrombocytopenia is seen in congenital amegakaryocytic thrombocytopenia (autosomal recessive), thrombocytopenia with absent radii (variable inheritance), and thrombocytopenia with radioulnar synostosis (autosomal dominant). Unlike other inherited thrombocytopenias, infants with thrombocytopenia-absent radius syndrome can demonstrate spontaneous resolution of thrombocytopenia during childhood. Although supportive care with platelet transfusions commonly is used as initial management for patients with inherited thrombocytopenia, accurate diagnosis is important because some conditions are associated with an increased risk of leukemia and some may benefit from bone marrow transplant.

Coagulopathy

Newborns

Accurate assessment of hemostasis in the newborn requires knowledge of the normal range for coagulation parameters (Table 2-7). The vitamin K-dependent factors II, VII, IX, and X and contact factors are physiologically low in neonates, despite the routine administration of vitamin K. Notably, the normal newborn range for factor IX activity, 15% to 50%, occasionally has led to the misdiagnosis of mild hemophilia B. By contrast, several factors are at adult levels at birth, including factors VIII, V, and XIII; fibrinogen; and VWF. Because of these physiologic differences, both the median and upper limit of PT (median, 16.7 seconds; upper limit, 23.5 seconds) and aPTT (median, 44.3 seconds; upper limit, 52 seconds) are higher than ranges established for adult patients. Coagulation factor production gradually increases over the first few months of life, reaching adult levels by approximately 6 months of age. Therefore, comparison of obtained values to age-appropriate normal values is a critical first step in evaluation of a neonate with suspected coagulopathy.

In sick neonates, coagulation abnormalities can result from sepsis, asphyxia, or other triggers of DIC. Unexpected bleeding in an otherwise well newborn, such as hemorrhage at circumcision, prolonged oozing from heel-stick blood draws, umbilical cord bleeding, or more bleeding or bruising than expected from a difficult delivery, should raise the possibility of an inherited bleeding disorder. Screening can be undertaken for PT and aPTT, with specific factor levels based on results and clinical concern.

The most common inherited causes of an isolated aPTT in an otherwise healthy infant are factor VIII and factor IX deficiency, with factor XI deficiency being significantly less common. Family history may be suggestive of a bleeding disorder with X-linked inheritance; however, a negative family history does not exclude the diagnoses, as approximately one-third of infants represent spontaneous mutations. Although also sometimes associated with an elevated aPTT, von Willebrand disease (VWD) rarely results in bleeding in the newborn unless it is severe (type 3). If there is an immediate need for treatment and the specific factor deficiency is unknown, FFP will provide adequate hemostatic coverage; however, it is important to draw a sample for specific factor testing prior to administration of FFP.

Elevation of both the PT and aPTT should prompt investigation of global defects in hemostasis such as vitamin K deficiency. Although all infants born in the hospital should receive supplemental vitamin K, home deliveries and parental desire to avoid medical interventions have increased the incidence of vitamin K deficiency in breast-fed infants. Vitamin K deficiency may be classified as early (within the first 24 hours of life), classic (between days of life 2 through 7), or late (beyond day 8 of life and as late as 6 months). Late deficiency is associated with a higher rate of intracranial hemorrhage. Infants often present with diffuse severe hemorrhage that can be intracranial, gastrointestinal, umbilical, head or neck, at injection sites, or from circumcision. Treatment for infants with mild bleeding is administration of 1 to 2 mg of vitamin K given either subcutaneously or as slow intravenous infusion. Rapid reversal of the coagulopathy begins within an hour of administration, but FFP should be given to infants with severe bleeding. Additional defects that affect global hemostasis include DIC and liver disease. Alternative causes of prolongation of both the PT and aPTT include rare deficiencies in factors of the common pathway, such as afibrinogenemia or dysfibrinogenemia, prothrombin deficiency, and factor V and factor X deficiency.

If there is a high suspicion of a bleeding disorder, and both the PT and aPTT are normal, factor XIII deficiency should be considered. This condition is an autosomal recessive disorder caused by an inability to cross-link fibrin and commonly presents with umbilical cord bleeding. A factor XIII activity is used to confirm the diagnosis. As mentioned above, VWD variably presents with a prolonged aPTT, and cannot be excluded with normal screening labs. However, most VWD does not present with bleeding in the neonatal time period, and the most severe will typically be accompanied by low factor VIII and therefore a prolonged aPTT. An additional concern in a bleeding patient with

normal screening labs would be a rare platelet function defect, such as Glanzmann's thrombasthenia.

Children

The diagnostic workup for a child with a suspected coagulopathy begins with a thorough history and screening, with a complete blood count, PT and aPTT. Specific considerations for additional testing depend on concerns identified on history and screening laboratory examination. Samples should be drawn from a peripheral venipuncture in order to avoid contamination from heparin. Here we provide an overview to guide the initial evaluation based on laboratory findings, with more specific information on individual disorders of coagulation in Chapter 10.

If an abnormality is identified, laboratory error or heparin contamination should be considered and eliminated as a possible cause. A lupus anticoagulant should be ruled out as described in the above section on perioperative bleeding. Patients with a concerning history should be evaluated for a factor deficiency. The child may have a remote history of bleeding, such as hemorrhage with circumcision, hematomas with immunizations, swelling to extremities with mild trauma, or previous bleeding with even minor procedures. Family history may provide information to guide testing, with factor VIII and IX deficiency having an X-linked inheritance. Testing for factor VIII and factor IX deficiency as well as VWD should be considered in children with a prolonged aPTT. Very rarely, factor XI deficiency can result in a prolonged aPTT and should be tested if no other abnormalities are identified. Mild hemophilia and VWD may not result in a prolonged aPTT. Therefore, specific factor testing should be undertaken if a high clinical suspicion exists.

An isolated prolonged PT usually represents a deficiency of factor VII. Inherited factor VII deficiency is a rare autosomal bleeding disorder with variable presentation and little correlation between bleeding rates and factor level. Beyond congenital factor VII deficiency, consideration should be given to acquired causes of factor VII deficiency such as liver disease and vitamin K deficiency from malabsorption, cystic fibrosis, or medication use. Given the extremely short half-life of factor VII, the PT may prolong before the aPTT.

Prolongation of both the PT and aPTT is seen in either common pathway factor deficiencies or in the setting of multiple factor abnormalities. Common pathway factor deficiencies are rare and include deficiencies of fibrinogen, prothrombin, factor V, and factor X. More commonly, this scenario is seen with multiple factor deficiencies in the setting of liver disease, vitamin K deficiency, and DIC. Testing of a combination of factors, such as factors VIII, V, and II,

often can provide information to distinguish these etiologies if they are not clinically apparent. In DIC, all 3 will be decreased; in liver disease, factor VIII will remain normal or elevated; and in vitamin K deficiency, only factor II will be decreased.

In all cases, treatment should be aimed at reducing hemorrhage and correcting coagulopathy with management of the underlying disease and replacement of deficient factors. If the precise deficiency is identified, specific factor replacement should be provided; however, if a specific factor is not available, the deficiency is not known, or multiple factors are involved, then FFP can be given.

Thrombosis

Newborns

Similar to pregnancy, the balance between hemostasis and fibrinolysis is shifted toward thrombosis in the newborn, with antithrombin III (ATIII) levels being mildly lower in neonates and the vitamin K-dependent anticoagulants, proteins C and S, strikingly lower (Table 2-7). Although evidence suggests that the fibrinolytic system is activated at birth, plasminogen levels are relatively low, so plasmin generation is somewhat decreased in response to thrombolytic agents. When added to the physiologic stresses of labor and delivery, the newborn period thus represents the greatest risk of thrombosis, especially in the sick neonate. Neonatal thrombotic complications include those associated with umbilical venous or arterial catheters, renal vein thrombosis, arterial and venous stroke, and cerebral sinus venous thrombosis. Clinically, it may be difficult to determine whether the thrombotic event occurred pre- or postnatally. In particular, cerebral sinus venous thrombosis and renal vein thrombosis have a higher relative incidence in childhood.

Screening for inherited thrombophilia in a neonate with a first thrombotic event is controversial; although some recommend screening all such infants, others conclude that unless it will alter acute management, screening is not cost effective. In addition, in neonates, age-related variation in normal factor levels may complicate interpretation of results. Lastly, in some cases, the mother and/or infant may be screened for antiphospholipid antibodies, which can cross the placenta.

Special mention should be made of the rare but potentially devastating homozygous deficiencies of protein C and protein S. Infants classically present with purpura fulminans lesions at birth without an obvious other cause for DIC. The level of protein C or S in such patients is often undetectable. There have also been reports of compound

heterozygous cases. Genetic testing can be performed to confirm a congenital cause but should not delay immediate treatment with FFP, along with anticoagulation with LMWH or UFH. Anticoagulation can be transitioned to warfarin once therapeutic levels of LMWH or UFH are achieved. Protein C concentrates are approved for use in patients who have confirmed severe protein C deficiency. Generally, protein C or protein S replacement should be administered for 6 to 8 weeks, until all lesions have healed and a therapeutic INR has been achieved.

Beyond protein C and protein S deficiency, treatment in infants with acute thrombosis can include thrombolytic therapy, UFH, warfarin, and LMWH. Thrombolytic therapy can be considered in the newborn when thrombosis poses risk to life, limb, or organ function. Dosing of tissue plasminogen activator may be somewhat higher in newborns compared with dosing in older patients due to lower levels of plasminogen. UFH use may be complicated by low levels of ATIII in infants. Therefore, if it is difficult to achieve a therapeutic aPTT, ATIII levels can be checked and a supplement can be given if levels are low. Warfarin dosing in infants can be complicated by several factors, including changing levels of coagulation proteins in the first months of life, disparate levels of vitamin K in breast milk and fortified formulas, and lack of a liquid warfarin preparation. For these reasons, LMWH increasingly is preferred. Newborns have rapid metabolism of LMWH and thus higher starting doses are recommended in this population, and dose adjustments should be made as needed to maintain anti-Xa activity levels of 0.5 to 1 U/mL 4 hours after administration.

Children

Recent evidence suggests that thrombosis in children is becoming a more common event, perhaps because of the increased use of central venous catheters, greater recognition, or improved imaging techniques. For the most part, children with thrombosis have an identifiable secondary cause such as infection or central venous catheter, and spontaneous thrombosis is less common. Testing for thrombophilia in children with thrombosis or family history of it remains controversial; however, testing is generally recommended for children with spontaneous thrombosis. There are insufficient data to guide recommendations for routine testing in children with an acquired risk factor such as a central catheter. If desired, comprehensive testing includes protein C, protein S, and ATIII levels along with factor V Leiden and prothrombin G20210A gene mutations. Additionally, one should consider lupus anticoagulant and antiphospholipid antibody testing in a child without other causes for spontaneous thrombosis. *MTHFR* mutational

analysis and homocysteine testing have been largely abandoned due to unclear significance. The rationale for testing is based on the notion that identification of thrombophilia may alter duration of anticoagulation therapy and predict risk for recurrence. Treatment for children with thrombosis is similar to adults, and duration is based on the site and cause of thrombosis (see Chapter 9).

Given that spontaneous thrombosis is rare in children, when it does occur, specific consideration should be given to anatomical causes. May-Thurner syndrome caused by pressure on the left common iliac vein by an overlying right common iliac artery should be suspected in cases of left iliac vein thrombosis and evaluated with an MRI once acute obstruction has resolved. Paget-Schroetter syndrome results from upper venous obstruction seen with thoracic outlet syndrome. Patients may report activity that requires frequent movement that raises the arm above the head leading to repeat compression. If present, proper management of both conditions involves consultation with a vascular surgeon or interventional radiology.

KEY POINTS

- Attention must be given to age-appropriate normal values when performing a pediatric consult.
- The sick newborn is particularly at risk for developing cytopenias secondary to poor bone marrow reserve in the setting of stress.
- Ideal prophylactic transfusion thresholds for red cell and platelet transfusions in neonates and children remain unknown.
- During the newborn period, antigenic differences between the mother and the infant can result in alloimmune cytopenias.
- The majority of hematologic conditions during childhood represent benign self-limited conditions and inherited causes are rare.
- ITP in children, unlike in adults, usually is acute, and management with observation alone is appropriate only for children with ITP and cutaneous manifestations.

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Consultative hematology II: women's health issues

PETER A. KOUIDES AND MICHAEL PAIDAS

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The role of a multidisciplinary team in managing women with blood disorders

The diagnosis and management of women's health issues in hematology require a multidisciplinary approach involving some combination of hematologists, internists, family practice physicians, obstetrician-gynecologists, pediatricians, surgeons, anesthesiologists, and other health care providers. Because women and girls with blood disorders may be at greater risk for bleeding, thrombosis, and reproductive pregnancy complications, their care requires a team of experts with the availability of specialized laboratory, pharmacy, and blood bank support (Table 3-1). For obstetrical care, this team should also include a maternal fetal medicine (MFM) specialist, because in many academic centers it is the MFM group that consults the hematologist, and some of these patients require shared care between MFM and hematology.

Whether the patient is an adolescent, pregnant, or a perioperative or critically ill female, or whether the setting is inpatient, outpatient, or phone consultation with a nearby emergency room or hospital, the role of a hematologist specifically trained in women's health issues is essential to ensure optimal

Conflict-of-interest disclosure: Dr. Kouides: coprincipal investigator for Octapharma-sponsored, investigator-initiated trial in postpartum hemorrhage; member of the National Blood Clot Alliance Medical & Scientific Advisory Board; member of the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation and a member of the Medical Advisory Committee of the Foundation for Women and Girls with Blood Disorders. Dr. Paidas: receives research funding from CSL Behring as principal investigator for postpartum hemorrhage associated with placenta accreta treatment trial; has received research funding from rEVO Biologics and was principal investigator for a preterm preeclampsia treatment trial; receives research funding from BioIncept LLC; receives grant funding from GestVision as a principal investigator for a preeclampsia prediction study; receives grant funding from Progenity as a principal investigator for a preeclampsia prediction study. Dr. Paidas is a member of Scientific Advisory Board of BioIncept LLC and has stock options. He is a member of the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation and a member of the Medical Advisory Committee of the Foundation for Women and Girls with Blood Disorders.

Off-label drug use: Dr. Kouides: clotting factor concentrates (plasma and recombinant), cyclosporine, desmopressin, eculizumab, eltrombopag, erythropoietin, gammaglobulin, romiplostim, and tranexamic acid in pregnancy. Dr. Paidas: aspirin, fondaparinux, low-molecular-weight heparin, and warfarin in pregnancy.

Table 3-1 Examples of the effect of multidisciplinary team care

Refinement of management plans during labor and/or delivery	Merging of expertise	Improved professional collaboration and team communication
Creation of individual patient care plans, with real-time documentation into the electronic health record	Capitalizes on the distinct perspectives and training of hematologists, obstetricians, and other specialists (anesthesia, neurology, cardiology, pediatrics)*	In-person meetings increase familiarity, mutual respect, and candor
Decisions on timing of anticoagulation interruption and restart based on expected obstetric course	Allows modification of treatment plans based on information that other teams impart	Timely patient care results from continuous collaboration
<i>Example: Anticoagulation planning for a woman with a third trimester PE makes allowances for expected labor timeline (ie, is she nulliparous, or has she had 3 prior, rapid deliveries), and/or mode of delivery.</i>	<i>Example: Timing of the transition from LMWH to UFH for VTE prophylaxis (ie, in a woman with no preterm birth risks vs a woman carrying twins, or with a history of preterm birth at 32 wk).</i>	<i>Example: A first obstetric visit for a woman with a complex thrombosis history: a telephone call made to the hematology team results in immediate shared decisions on care, without waiting for a formal consultation.</i>

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*At the authors' institution, they have a quarterly conference between obstetric care providers and hematologists, with review of shared patients kept on a continuously updated list. Members of other disciplines are asked to join the meeting when needed. In-person discussions of patient care plans are scheduled outside of these quarterly conferences on an as-needed basis, and frequently involve multiple disciplines.

outcomes. Furthermore, the plan of care should be formulated with the multidisciplinary team, when available, utilizing evidence-based guidelines from expert panels of the American College of Obstetrics and Gynecology (ACOG); American College of Chest Physicians (ACCP); Council on Patient Safety in Women's Health Care (<http://safehealthcareforeverywoman.org/>); Foundation for Women and Girls with Blood Disorders; National Heart, Lung, Blood Institute (NHLBI); National Hemophilia Foundation (NHF); National Partnership for Maternal Safety; and the World Health Organization (WHO). Collaboration is critical from the beginning to the end of care, including awareness among caregivers and proficiency for early diagnosis, and the development of an antepartum surveillance plan and a peripartum plan specifying the details and duration of treatment and assigning respective responsibilities for each part of the plan. This plan of care should be communicated in a timely manner with all consulting care providers, as well as the patient.

This chapter summarizes the most recent evidence and guidelines available to minimize risk in the woman with blood disorders, in particular in the pregnant woman and the premenopausal female. The hematologist may play a critical role (directly or indirectly) in the care of such patients, in a number of scenarios—whether serving on hospital committees, working groups, or formulary committees; or developing clinical practice guidelines, establishing policies and procedures for transfusion services, monitoring quality of care and service efficiency, or consulting

for the federal government or pharmaceutical industry. Although these latter roles are not addressed specifically in this chapter, the data management, organizational, and communication skills required for providing patient care or consultation are just as critical as those required when working in advisory groups. The clinical hematologist also serves patients well when adhering to the principles of effective communication in work with other physicians and consultants, house staff, fellows, students, and the patient and family. A commitment to effective multidisciplinary team collaboration and communication ensures the highest quality of patient care and optimal patient outcomes.

Hematologic health issues in pregnancy

Anemia in pregnancy

During normal pregnancy, the plasma volume expands by 40% to 60%, whereas the red blood cell mass expands by 20% to 50%. Based on this dilutional effect, according to ACOG and the Centers for Disease Control and Prevention (CDC), anemia is defined as a hemoglobin value of <11 g/dL or hematocrit <33% in the first trimester, hemoglobin value of <10.5 g/dL or hematocrit <32% in the second trimester, hemoglobin of <11 g/dL or hematocrit <33% in the third trimester; although a 10.8 g/dL hemoglobin cutoff has been proposed for African Americans. Hemoglobin levels of <10 g/dL suggest the possibility of a pathologic process, such as nutritional deficiency. The

prevalence of anemia in pregnancy increases from 8% in the first trimester to 12% in the second trimester and 34% in the third trimester. At present, there is no definitive evidence whether the hemoglobin threshold for transfusion should be <7 or <8 g/dL, although the increased fetal oxygenation needs entering the third trimester and the increased oxygenation needs of labor and the risk of excess blood loss prompts most experts to raise the threshold to 8 g/dL in the third trimester. On the other hand, avoiding transfusions is ideal, particularly given the risk of red cell antigen sensitization and the risk of hemolytic disease of the newborn in subsequent pregnancies. The main determinant to transfuse should be the presence of active hemorrhage or hemodynamic compromise but there should be consideration also of the patient's preference and symptomatology and alternative therapies.

Iron deficiency anemia

Iron deficiency accounts for 75% of cases of nonphysiologic anemia in pregnancy, and the incidence of iron deficiency anemia in the United States during the third trimester may exceed 50%. Clinical manifestations of iron deficiency include fatigue, tachycardia, dyspepsia, poor exercise tolerance, and suboptimal work performance. In addition, iron deficiency is associated with postpartum depression, poor maternal-infant behavioral interaction, impaired lactation, low birth weight, premature delivery, intrauterine growth retardation, and increased fetal and neonatal mortality. The risk for iron deficiency anemia of pregnancy includes multiparity, short recoveries between pregnancies, poor nutritional status, and poor socioeconomic status. The total iron requirement during pregnancy is 1,190 mg, and with a net iron balance during pregnancy of 580 mg, this equates to a requirement of 2 mg daily. Even with a normal diet, this is hard to maintain. Besides poor nutrition, other factors impairing iron absorption include antacids and micronutrient deficiencies—including vitamin A, vitamin C, zinc, and copper. In the absence of iron supplementation, hemoglobin falls to 10.5 g/dL at 27 to 30 weeks of gestation; with iron supplementation, the nadir is less severe, 11.5 g/dL. By the third trimester, serum ferritin declines, erythropoietin levels surge, and maternal hepcidin levels are reduced to facilitate iron transfer and use at delivery.

Current recommendations suggest that pregnant patients receive 30 mg daily of supplemental elemental iron, although studies examining the efficacy of iron supplementation during pregnancy have not shown a clear benefit in terms of pregnancy outcomes. Ferrous gluconate or polysaccharide-iron (Feramax or Ferrex) are better tolerated due to fewer gastrointestinal effects than ferrous sulfate.

For patients who do not tolerate oral iron (in general, up to 70%), parenteral iron may be used. While the U.S. Food and Drug Administration does not specifically restrict the use of parenteral iron preparations in the first trimester, the European Medicine Agency's Committee of Medicinal Products for Human Use does. Iron sucrose is categorized as pregnancy class B (presumed safe based on animal models) and is preferred over iron dextran, iron fumoxitol, or ferric carboxymaltose, which are considered pregnancy class C (safety uncertain) though this categorization reflects the fact that there are fewer data in humans about these preparations. However, there are emerging data for the safety and efficacy of ferric carboxymaltose as a one-time infusion of 1,000 mg, as opposed to giving several 200 to 400 mg doses of iron sucrose because unlike iron sucrose, there is greater binding of the carbohydrate moiety to iron. With parenteral iron, the hemoglobin increase in 28 days ranges from 1.3 to 2.5 g/dL compared with a 0.6 to 1.3 g/dL increase with oral iron.

In the rare situation that the patient does not respond to parenteral iron, another option may be the addition of recombinant erythropoietin (rEPO). rEPO does not cross the placenta. Although rEPO may function as an adjuvant to iron replacement therapy in pregnant patients with iron deficiency anemia, it should be reserved for exceptional cases, given the heightened prothrombotic state of pregnancy and the fact that improved fetal outcomes have not been demonstrated. Alternative causes of anemia should be sought in patients refractory to standard iron therapy. Finally, although iron supplementation improves hematologic parameters, it may not improve neonatal outcomes.

Recommendations. For pregnant women, daily 30 mg elemental iron is recommended. If anemia develops, daily or alternate dosing as opposed to daily divided dosing is advised. Concurrent vitamin C (250 mg) may increase iron absorption. For those not able to tolerate oral iron, parenteral iron after the 13th week is appropriate; iron sucrose is preferred but ferric carboxymaltose can be considered.

Megaloblastic anemia

The majority of macrocytic anemias during pregnancy are due to folate deficiency, whereas vitamin B₁₂ deficiency is rare but is seen more often in the United States given the increased frequency of bariatric surgery with the Roux en Y technique, which can lead to vitamin B₁₂ and iron deficiency. The clinician should be reminded that the mean cell volume in pregnancy may be normal in B₁₂ or folate deficiency because it can be "masked" by concurrent iron deficiency or thalassemia trait. A physiologic decline in B₁₂ levels of 20% occurs in pregnancy but does not appear to be a true deficiency because the metabolites homocysteine

and methylmalonic acid are normal. Multivitamin and folic acid supplementation reduce the risk of placental abruption and recurrent pregnancy loss. Folate requirements increase from 50 µg daily in the nonpregnant female to at least 150 µg daily during pregnancy, and the CDC recommends supplementation with 400 µg daily of folate to prevent neural tube defects. Folate deficiency is most precisely diagnosed by measuring plasma levels of homocysteine and methylmalonic acid.

Recommendations. Homocysteine and methylmalonic acid testing should be done in cases of borderline B₁₂ deficiency (200 to 300 pg/mL). For pregnant women, daily folic acid 400 µg is recommended. A much higher dose of 5 mg, to begin 2 months before conception and continue during the first trimester (until closure of the neural tube), is needed in women with hemolytic disorders like sickle cell anemia or autoimmune hemolytic anemia; as well as women who are at high risk of having offspring with neural tube defects. This includes women with certain folate-enzyme genotypes and women with previous pregnancies with neural tube defects. Also, 5 mg dosing is advised for women who smoke or who have diabetes, malabsorption disorders, obesity, or exposure to antifolate medications within 2 months of conception (eg, methotrexate; sulfonamides; antiepileptics like carbamazepine, valproate, barbiturates). After the first trimester, the folic acid dose can be reduced as the safety of long-term, high-dose folate supplementation in pregnancy is unknown.

Aplastic anemia

Aplastic anemia is rare in pregnancy. It may be either associated with or precipitated by pregnancy. Some cases may either mimic or occur with immune thrombocytopenic purpura (ITP). The mechanism of the bone marrow aplasia that occurs in pregnancy is believed to be through the erythropoietic suppressor effects of hormones during pregnancy. Alternatively, preexisting aplasia may be uncovered during pregnancy. In a recent single institution study of 24 pregnancies in 24 years, there were no maternal deaths but a high rate of complications in 80%—including transfusion and drug-related events, bleeding, infection, preterm birth, and thrombosis. Unfortunately, stem cell transplantation, which is the major therapy for nonpregnant aplastic anemia, is contraindicated in pregnancy. Women with preexisting aplastic anemia have a better prognosis than those with pregnancy-induced aplastic anemia, although the treatment is similar, including transfusion to maintain a platelet count >20,000/µL, growth factors (eg, granulocyte colony-stimulating factor) and, in select cases, prednisone

and/or cyclosporine (Grade 2C recommendation of the 2015 British Society for Standards in Haematology). Antithymocyte globulin is not recommended. Among women who survive pregnancy-associated aplastic anemia, half may experience spontaneous remission, and the remainder are managed with antithymocyte globulin, immunosuppression, or stem cell transplantation.

Recommendations. For pregnant women with aplastic anemia, transfusions to maintain a hemoglobin of 7 to 8 g/dL, a platelet count of >20,000/µL (but >50,000/µL for delivery and >80,000/µL for epidural), and growth factors (eg, G-CSF), as needed, are recommended. In pregnancy-induced aplastic anemia, the role of termination or early delivery should be considered in management; case reports indicate improvement of aplastic anemia following pregnancy.

Autoimmune hemolytic anemias

Very few cases have been reported. There appears to be a higher risk of preeclampsia. The newborn is at a moderate risk for anemia. As in the nonpregnant patient, front-line treatment with prednisone applies; and in refractory cases, splenectomy (in the second trimester) or cyclosporine (with caveat as noted in preceding section) or rituximab (to be discussed in detail in the ITP section) can be considered.

Recommendations. Folic acid (5 mg) should be prescribed beginning 2 months preconception and continue until the end of the first trimester; then the dose can be reduced. Low-dose aspirin (LDA) should be given for preeclampsia prevention. Immunosuppressive therapy as noted above.

Microangiopathic hemolytic anemias

Microangiopathic hemolytic anemias are disorders characterized by hemolytic anemia in association with thrombocytopenia and multiorgan failure. Hemolysis is caused by microthrombi in small capillaries and characterized by schistocytes, elevated lactate dehydrogenase (LDH) and indirect bilirubin, and reduced haptoglobin. Although they represent an uncommon cause of anemia in pregnancy (estimates are >0.6% to 1% of pregnancies are complicated by microangiopathies), they may have devastating consequences for both mother and child. These disorders, which include thrombotic thrombocytopenic purpura (TTP); hemolytic uremic syndrome (HUS); preeclampsia; and hemolysis, elevated liver function tests, and low platelets syndrome (HELLP), are challenging to diagnose, given the wide overlap in clinical presentation, and difficult to treat, given disparate treatments. These are discussed in the section “Thrombocytopenia in pregnancy.” Recommendations are provided for each disorder.

Hereditary anemias excluding sickle cell anemia

Hereditary spherocytosis is relatively common among patients of Northern European descent. However, there is very little information about pregnancy. Pregnancy may precipitate or worsen hemolytic crisis, and maternal morbidity and fetal outcomes appear to be more favorable in previously prepartum splenectomized patients.

Regarding thalassemia, there is information regarding its course in pregnancy. Thalassemia minor and intermedia are associated with favorable outcomes, while beta thalassemia major can have a favorable outcome if (per ACOG guidelines) the patient has normal cardiac function and has had prolonged hypertransfusion therapy to maintain hemoglobin levels at 10 g/dL and iron chelation therapy.

Recommendations. In both hereditary anemias, a higher than standard dose of folic acid (5 mg) should be prescribed beginning 2 months preconception and continue until the end of the first trimester; then the dose can be reduced. In beta thalassemia major, the hemoglobin level should be maintained at or near 10 g/dL and chelation therapy should be stopped. Fetal growth should be periodically monitored. The mode of delivery should be based on obstetric indications. Genetic testing and counseling should be offered even to patients with thalassemia trait.

Sickle cell anemia

It is well established that pregnancy in women with sickle cell anemia is very high risk, related to underlying hemolytic anemia and multiorgan dysfunction. As oxygen demand increases to meet the requirements of the growing fetus and placenta, along with the expanding blood volume, red cell requirements increase. Further, pain crises and other complications may worsen if red cell production cannot keep up with oxygen demand. If possible, precipitating factors such as dehydration, stress, excessive exertion, and a cold environment should be avoided. The problems of oxygenation and pathophysiology of sickling may result in both maternal and fetal morbidity. The relative risk of maternal mortality is about 6-fold. In addition, preeclampsia, eclampsia, placental abruption, and antepartum bleeding may complicate pregnancy; and preterm labor, intrauterine growth restriction, and intrauterine fetal death may complicate gestation (4-fold risk of stillbirth). Nearly half of the women with sickle cell disease (SCD) require an acute transfusion due to severe anemia or obstetric emergency.

As already noted, while there is no specific transfusion trigger in pregnancy other than a hemoglobin <6 gm/dL, the goal in pregnant SCD patients is to maintain prepregnancy hemoglobin. A recent survey among maternal-fetal medicine experts in the United States and Canada showed

a wide variation in transfusion practice that reflects a 2016 Cochrane review, which concluded that there was inadequate evidence on whether prophylactic or selective transfusion is the best approach, let alone whether the prophylactic transfusion method should be simple transfusion, manual exchange, or automated exchange.

If pain crises escalate, more frequent or even regular (eg, monthly) transfusions may be required. Optimal management of other complications (eg, acute chest syndrome) may also require more frequent transfusion, as would those with a history of previous perinatal mortality. Maternal mortality risk is up to 10% in women with SCD pulmonary hypertension. The 2014 National Institutes of Health guidelines recommend discontinuing hydroxyurea in pregnancy and during breastfeeding, but few human data exist on potential harmful reproductive effects of hydroxyurea in males and females.

It is suggested that iron chelation therapy be discontinued preconception. Early in pregnancy, supplemental folic acid at 5 mg daily (a higher dose than standardly given in pregnancy) should be initiated. Alloantibody screening should also be performed early and, if positive, phenotypic matching should be considered to avoid delayed hemolytic transfusion reactions or hemolytic disease of the newborn. In addition, 10% or more of patients with SCD develop a venous thromboembolism (VTE) by adulthood, and increased risk is also typical in pregnancy. Contributing risk factors for VTE in SCD may include immobilization during hospitalization, vasoocclusive crisis, intravenous access devices, and chronic hemolysis. In one database of 18,000 deliveries, SCD was associated with higher rates of cerebral vein thrombosis and deep venous thrombosis (DVT) than the control group, while another database of 14,000 deliveries found an increased risk of VTE comparable to that of pregnant lupus patients.

Recommendations. The goal during pregnancy is to maintain prepregnancy hemoglobin and provide more frequent or regular transfusions for increasing pain crises or other complications (eg, acute chest syndrome [ACS]). As in nonpregnant patients, the indications for exchange transfusion (manual or automated) in lowering the sickle hemoglobin <30% would apply (ACS, secondary stroke prevention). Also, exchange transfusion can be considered in patients deemed to be at high risk of placental detachment at the time of acute vasoocclusive crisis. Alloantibody screening should be performed in early pregnancy, and in heavily immunized pregnant women, phenotypically matched products should be given if possible. Pregnant SCD patients with a prior history of VTE warrant antepartum and postpartum thromboprophylaxis (see section “Thromboembolism and thrombophilia in pregnancy” in

this chapter). For SCD patients without a past history of VTE, while there are no clinical trials, antepartum and postpartum low-molecular-weight heparin (LMWH) prophylaxis should be considered if there are prothrombotic risk factors such as immobilization (eg, hospitalization for vasoocclusive crisis) or obesity. Given the increased risk of preeclampsia, LDA should be strongly considered at the beginning of the second trimester until 5 to 10 days before the expected day of birth. Lastly, given the increased risk of neural tube defects with increased maternal turnover of folate, 5 mg of folic acid is advised to begin 2 months preconception and continue until the end of the first trimester; then the dose can be reduced.

Thrombocytopenia in pregnancy

After anemia, thrombocytopenia is the most common hematological abnormality of pregnancy. Thrombocytopenia affects approximately 10% of pregnant women and results from several disorders that may or may not be specific to pregnancy. Pregnant patients may present with isolated

thrombocytopenia or may develop thrombocytopenia as a component of a systemic disorder that may be unique to pregnancy. A summary of causes of thrombocytopenia in pregnancy is presented in Table 3-2. The distribution of the various thrombocytopenic conditions is depicted in Figure 3-1.

Importantly, unless the platelet count is rapidly falling or there is a concurrent bleeding risk like aspirin or anti-coagulant use or inherited platelet dysfunction, pregnant thrombocytopenic patients can be cleared for an epidural/spinal anesthesia if the platelet count is $>80,000/\mu\text{L}$ (Level C recommendation, ACOG practice bulletin, #166, September 2016). Patients with platelet counts $>50,000/\mu\text{L}$ but $<80,000/\mu\text{L}$ can be considered for a central neuraxial anesthesia after discussion with the patient, anesthetist and/or anesthesiologist and conferring hematologist. The platelet count cutoff for delivery ideally should be $>50,000/\mu\text{L}$. Though there are reports of vaginal delivery below $50,000/\mu\text{L}$, in case there is conversion to a cesarean section (CS), $>50,000/\mu\text{L}$ is advised. However, in

Table 3-2 Differential diagnosis of thrombocytopenia in pregnancy

Diagnosis	Severity of thrombocytopenia	MAHA defect	Coagulation defect	Hypertension	Liver disease	Renal disease	CNS disease	Time of onset
ITP	Mild to severe	—	—	—	—	—	—	Common in first trimester
Gestational	Mild	—	—	—	—	—	—	Second and third trimesters
Preeclampsia	Mild to moderate	Mild	Absent to mild	Moderate to severe	Absent to severe	Proteinuria	Seizures in eclampsia	Late second to third trimester and postpartum
HELLP	Moderate to severe	Moderate to severe	May be present (mild)	Absent to severe	Moderate to severe	Absent to moderate	Absent to moderate	Late second to third trimester and postpartum
HUS	Moderate to severe	Moderate to severe	Absent	Absent to mild	Absent	Moderate to severe	Absent to mild	Postpartum
TTP	Severe	Moderate to severe	Absent	Absent to severe	Absent	Absent to moderate	Absent to severe	Second to third trimester
AFLP	Mild to moderate	Mild	Severe	Absent to severe	Severe	Absent to mild	Absent to severe	Third trimester
DIC	Moderate to severe	—	May be severe	Absent	Absent	Absent	Absent	Third trimester
PNH	Moderate to severe	—	Absent	Absent	Absent	Absent	Absent	All trimesters

For the diagnosis of preeclampsia, proteinuria is defined as $\geq 0.3 \text{ g}$ in a 24-hour urine specimen or protein/creatinine ratio ≥ 0.3 (mg/mg), or dipstick $\geq 1+$ if a quantitative measurement is unavailable, according to American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Thrombocytopenia is mild when platelets are $>50,000/\mu\text{L}$, moderate when $>20,000/\mu\text{L}$, and severe when $<10,000/\mu\text{L}$.

AFLP, acute fatty liver of pregnancy; CNS, central nervous system; DIC, disseminated intravascular coagulation; HELLP, hemolysis, elevated liver function tests, low platelets; HUS, hemolytic uremic syndrome; ITP, idiopathic thrombocytopenic purpura; MAHA, microangiopathic hemolytic anemia; PNH, paroxysmal nocturnal hemoglobinuria; TTP, thrombotic thrombocytopenic purpura.

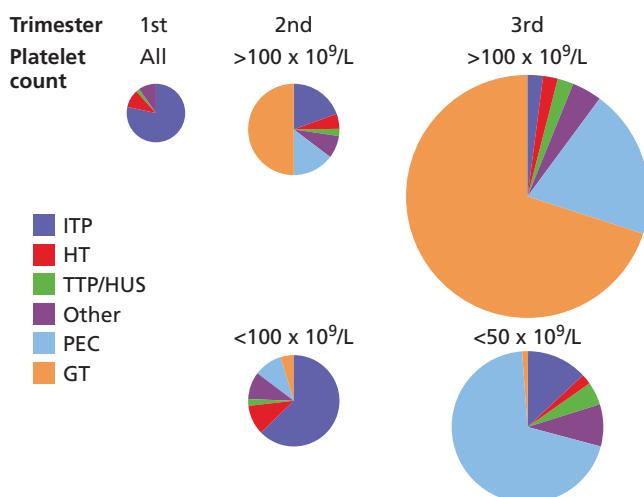


Figure 3-1 Distribution and timing of thrombocytopenic conditions in pregnancy. Prevalence of causes of thrombocytopenia based on trimester of presentation and platelet count. The size of each circle represents the relative frequency of all causes of thrombocytopenia during each of the 3 trimesters of pregnancy. All etiologies and all platelet counts are considered together in the first trimester when thrombocytopenia is uncommon. Distribution of etiologies during the second and third trimesters is subdivided by platelet count. All results are estimates based on personal experience and review of the literature. “Other” indicates miscellaneous disorders, including infection, DIC, type IIB von Willebrand disease, immune and nonimmune drug-induced thrombocytopenia, paroxysmal nocturnal hemoglobinuria, bone marrow failure syndromes (aplastic anemia, myelodysplasia, myeloproliferative disorders, leukemia/lymphoma, and marrow infiltrative disorders), among others. HUS, hemolytic uremic syndrome; PEC, preeclampsia/HELLP; TTP, thrombotic thrombocytopenic purpura. Redrawn from Cines DB, Levine LD, *Blood*. 2017;130(21):2271–2277, with permission.

both situations (central neuraxial anesthesia and delivery), it is acknowledged that local practice patterns vary and a discussion with the patient and multidisciplinary team is necessary.

Gestational thrombocytopenia

Isolated thrombocytopenia most commonly results from “gestational” or “incidental” thrombocytopenia of pregnancy. Gestational thrombocytopenia (GT) occurs in 4% to 12% of all pregnancies, usually during the second or third trimester, and rarely in the first trimester in otherwise healthy pregnant women. It is defined as a platelet count below $150,000/\mu\text{L}$. Thrombocytopenia is usually mild and self-limited, requiring no treatment, and typically does not decrease below $70,000/\mu\text{L}$, but on occasion, gestational thrombocytopenia with platelet counts below $50 \times 10^9/\text{L}$ have occurred. The mechanism of gestational thrombocytopenia is not well established. It appears to be

multifactorial—including the dilutional effect of pregnancy, pooling or consumption of platelets in the placenta, heightened immunological destruction, or increased macrophage colony-stimulating factor from the placenta. There is no diagnostic test for gestational thrombocytopenia, so it is a diagnosis of exclusion. There are several salient features: (1) onset as noted in the mid-second to third trimester; (2) asymptomatic with no prior history of bleeding; (3) no effect on pregnancy outcome and does not result in thrombocytopenia in the offspring of affected women; (4) it is usually self-limited and resolves 4 to 8 weeks postpartum but may recur to the same degree in subsequent pregnancies. As gestational thrombocytopenia may not be distinguishable from ITP or more serious disorders in late pregnancy, however, women with gestational thrombocytopenia should be monitored throughout pregnancy and the clinician should continue to track the platelet count every 2 to 4 weeks. A fall in the platelet count $<70,000/\mu\text{L}$ in the third trimester would usually be reclassified as ITP or preeclampsia/eclampsia and managed accordingly, because gestational thrombocytopenia can be viewed as being part of a continuum. For example, a subset of gestational thrombocytopenia may have a more pronounced decline in the platelet count associated with a reduced antithrombin III (ATIII) level along the lines of hemolysis, elevated liver enzymes, HELLP syndrome, and acute fatty liver of pregnancy (AFLP), which may be associated with a higher risk of recurrence in subsequent pregnancies.

Recommendations. No treatment is recommended, as the disorder generally resolves postpartum. Importantly, such patients can be cleared for central neuraxial anesthesia if the platelet count is $>80,000/\mu\text{L}$, per the ACOG practice bulletin. If the platelet count in the third trimester is falling to the $80,000/\mu\text{L}$ range, on the presumption that there may be a component of immune destruction, a course of low-dose prednisone (10 to 20 mg/day) can be considered in hopes of maintaining the platelet count $>80,000/\mu\text{L}$ to allow for the option of an epidural.

Immune thrombocytopenic purpura

ITP affects approximately 1 in 10,000 pregnancies. In contrast to gestational thrombocytopenia, ITP is usually detected in the first trimester. The diagnosis is a clinical one because antibody testing lacks specificity. A prior history of thrombocytopenia or autoimmune disease preceding pregnancy is useful in making a diagnosis of ITP. Patients with ITP generally present with more severe thrombocytopenia than those with gestational thrombocytopenia, but the 2 disorders may be indistinguishable when ITP is mild.

Only a third of patients require treatment of ITP during pregnancy. Indications for treatment of ITP in pregnancy

in the first 2 trimesters include: (1) when the patient is symptomatic, (2) when platelets fall $<30,000/\mu\text{L}$, or (3) to increase platelet count to a level considered safe for procedures. Although the lowest platelet count safe for central neuraxial anesthesia is controversial, as noted above most obstetric anesthetists recommend a platelet count of $80,000/\mu\text{L}$, and most hematologists recommend for at least $50,000/\mu\text{L}$ for CS delivery.

Therapy of ITP in pregnancy is similar to that in patients who are not pregnant. Corticosteroids and intravenous immunoglobulin (IVIg) are the first-line treatments for maternal ITP. Prednisone is usually given at a dose of 0.5 to 1.0 mg/kg/day, with adjustment to the minimum dose providing a hemostatically effective platelet count. Although short-term prednisone is considered effective and safe in the mother, it may exacerbate hypertension, hyperglycemia, osteoporosis, weight gain, and psychosis; and in the fetus, may increase the incidence of cleft palate if exposure is in the first trimester. The risks of IVIg, including infusion reactions with fever, rigors, headache (which can also be delayed in terms of aseptic meningitis), and renal complications with certain brands, should be discussed with the pregnant patient. Hematological risks include hemolytic anemia, neutropenia, and thromboembolic events. Rarely, there is a risk of anaphylaxis and a theoretical risk of transmission of bloodborne diseases.

Intravenous anti-D has been used successfully to treat ITP in Rh(D)-positive women, although data from only a few patients have been reported, and thus the safety of this agent cannot be considered established. Similarly, there is little experience with the use of rituximab, which is considered pregnancy class C, in pregnant individuals; B-cell lymphocytopenia has been reported in the offspring of individuals treated with this agent, wherein newborn vaccination would need to be delayed. The thrombopoietic agents romiplostim and eltrombopag also are considered pregnancy class C; a registry has been developed for patients taking these agents who become pregnant. The use of cytotoxic therapy is associated with teratogenicity in many cases, although azathioprine commonly has been used in pregnancy with apparent safety.

Up to 10% of the offspring of patients with ITP also are thrombocytopenic, and 5% have platelet counts $<20,000/\mu\text{L}$. There are no maternal laboratory studies that reliably predict whether an infant of a mother with ITP will be born thrombocytopenic; perhaps the best indicator is a prior history of thrombocytopenia at delivery in a sibling. Moreover, no maternal interventions have been shown convincingly to increase the fetal platelet count. The delivery of the offspring of mothers with ITP by CS has not been shown to reduce the risk of fetal intracranial hemorrhage,

a rare complication affecting <1% of these infants at delivery; however, some continue to advocate this approach, particularly when a sibling previously has been found to be severely thrombocytopenic at delivery. These considerations, and appreciation that the risk of bleeding with fetal platelet count determination by percutaneous umbilical cord blood sampling (PUBS) is greater than that of fetal intracranial hemorrhage during vaginal delivery, explain the abandonment of PUBS in recent years.

Management of ITP antepartum. For pregnant women, prednisone or IVIg is recommended for severe ITP. In those with severe ITP refractory to steroids and IVIg, splenectomy should be considered, optimally in the second trimester, when the risk of inducing premature labor is minimized and the gravid uterus does not yet obscure the surgical field. Nonsurgical options for refractory cases would include rituximab, thrombopoietic agents, and cyclosporine, as mentioned in the aplastic anemia and autoimmune hemolytic anemia sections.

Management of ITP peripartum. All offspring of patients with ITP should be monitored closely for the development of ITP within the first 4 to 7 days after delivery, and all thrombocytopenic neonates should undergo cranial ultrasound. For severely affected offspring, IVIg is recommended. Postpartum hemorrhage (PPH) can be noted in up to a quarter of ITP patients at a median platelet count of $\sim 60,000/\mu\text{L}$ compared to $\sim 130,000 \mu\text{L}$ in non-ITP patients. Consequently, consideration of prophylactic tranexamic acid (TXA) postpartum should be given, particularly if the platelet count is $<50,000/\mu\text{L}$ and certainly given if PPH ensues if not given prophylactically.

Preeclampsia and eclampsia

Thrombocytopenia also may occur in patients with preeclampsia. Preeclampsia affects 5% to 8% of all pregnancies and usually develops in the third trimester. Preeclampsia and its early form (preeclampsia occurring before 37 weeks' gestation) are increasing in prevalence. While hypertension is a key feature of preeclampsia, proteinuria is no longer considered an essential diagnostic criterion. Proteinuria is defined as proteinuria $>300 \text{ mg}/24 \text{ h}$, urine protein/creatinine ratio ≥ 0.3 , or dipstick $\geq 1+$ if a quantitative measurement is unavailable. Significant risk factors for preeclampsia include nulliparous women, extremes of maternal age ($<20 \text{ y}, >35 \text{ y}$), prior history of preeclampsia, multifetal gestation, chronic hypertension, diabetes mellitus, autoimmune disease, possibly antiphospholipid antibody syndrome, sickle cell disease, renal disease, obesity, infertility, family history of preeclampsia (mother or sister), and limited sperm exposure. Several genes regulating such diverse processes of metabolism, cell communication, and

immunity, as well as other processes, have been associated with preeclampsia.

The classification of hypertensive disorders of pregnancy was first introduced in 1972 by ACOG and modified in 1990, 2000, and most recently in 2013. Hypertensive disorders are classified as: preeclampsia-eclampsia, chronic hypertension (of any cause), chronic hypertension with superimposed preeclampsia, and gestational hypertension. Preeclampsia is further classified as preeclampsia with or without severe features. Any one of the following criteria fulfill criteria for preeclampsia with severe features: systolic blood pressure ≥ 160 or diastolic blood pressure ≥ 110 mm Hg on 2 occasions, at least 4 h apart while the patient is on bedrest (unless antihypertensive therapy is initiated before this time); thrombocytopenia (platelet count less than 100,000/ μ L); impaired liver function as indicated by abnormally elevated ALT or AST (to twice normal concentration), severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnoses, or both; renal insufficiency (serum creatinine concentration > 1.1 mg/dL, or doubling of serum creatinine in absence of other renal disease; pulmonary edema; new onset cerebral or visual disturbances.

Eclampsia, defined by the presence of grand mal seizures accompanying preeclampsia, complicates <1% of preeclamptic pregnancies. Up to 50% of patients with preeclampsia develop thrombocytopenia, the severity of which generally is related to that of the underlying disease. The pathogenesis of thrombocytopenia in preeclampsia is not well understood, but it has been hypothesized that a hypoxic placenta releases antiangiogenic factors, including soluble Flt-1 and soluble endoglin, which impair capillary angiogenesis, leading to endothelial dysfunction; and the clinical features of preeclampsia may evolve in response to endothelial dysfunction. The levels of sFlt1 and soluble endoglin in pregnant women are predictive of the severity of preeclampsia.

The observation that endothelial dysfunction and platelet dysfunction occur in preeclampsia has led to studies of antiplatelet agents, primarily LDA, in women with preeclampsia. In a Cochrane review of 43 randomized trials including over 32,000 patients, antiplatelet agents significantly reduced preeclampsia in both women at low and high risk for preeclampsia, if started before 20 weeks' gestation. Although the use of antithrombotic therapy, primarily LMWH, has been suggested by some for management of patients at high risk for preeclampsia in subsequent pregnancy (those with past preeclampsia, a body mass index [BMI] of > 35 kg/m 2 , preexisting diabetes, twin pregnancy, family history of preeclampsia, chronic hypertension, renal disease, autoimmune disease, or an

underlying angiotensin-converting enzyme insertion or deletion polymorphism), the preponderance of the literature (and especially from higher quality studies) suggests that heparin anticoagulation does not improve pregnancy outcome in subsequent pregnancy and should not be routinely prescribed.

Finally, disseminated intravascular coagulation (DIC) also may accompany severe preeclampsia and may be initiated by such processes as retained fetal products, placental abruption, or amniotic fluid embolism. In these settings, DIC can be severe, abrupt, and fatal if not managed appropriately.

Recommendations. At the present time, the threshold for LDA has been lowered and for women with a history of early onset preeclampsia and preterm delivery at $< 34^{0/7}$ weeks gestation or preeclampsia in more than 1 prior pregnancy, LDA beginning in the late first trimester should be considered.

Magnesium sulfate intrapartum and postpartum for seizure prophylaxis is given by some obstetricians in all cases of preeclampsia. There is a role for withholding magnesium sulfate in a select group of preeclamptic patients (systolic blood pressure of less than 160 mm Hg and a diastolic blood pressure less than 110 mm Hg and no maternal symptoms), and is supported by an ACOG guideline, but evidence supporting this position is of low quality.

Women who have delivered preterm with preeclampsia or have had recurrent preeclampsia are at increased risk for cardiovascular disease, namely chronic hypertension, thromboembolism and diabetes (metabolic syndrome); and annual screening of blood pressure, lipids, fasting glucose, and BMI, along with lifestyle modification and early intervention, are recommended.

Unless severe disease is present, delivery is indicated at 37 weeks of gestation.

Hemolysis, elevated liver function, low platelets

HELLP syndrome affects 0.10% to 0.89% of all live births and is associated with a maternal mortality rate of 0% to 4%. HELLP and preeclampsia share many clinical features, although HELLP occurs in a slightly older population (mean age 25 years). It occurs predominantly in the third trimester, between 28 and 36 weeks of gestation, and in some cases may occur postpartum, with up to 30% presenting within 48 hours of delivery, and even as late as 1 week postpartum. Generalized edema precedes the syndrome in more than 50% of cases. Approximately 70% to 80% of patients with HELLP also have preeclampsia, which by definition has hypertension plus/minus proteinuria. The major diagnostic criteria for HELLP include microangiopathic hemolytic anemia, levels of serum aspartate aminotransferase exceeding 70 U/L, and thrombocytopenia with

a platelet count < 100,000/ μ L. Microangiopathic hemolytic anemia is accompanied by schistocytes on the peripheral blood film and an elevated LDH; some experts suggest that a minimal LDH of 600 U/dL is required for diagnosis. In some cases, HELLP may be difficult to distinguish from TTP-HUS. Because many patients with HELLP may present with isolated right upper-quadrant and epigastric pain in the absence of hypertension and proteinuria, patients may be misdiagnosed as having primary gastrointestinal disease and referred for surgical consideration. HELLP is associated with significant maternal and fetal morbidity and mortality; therefore, prompt diagnosis and treatment are essential.

In general, if there is maternal hemodynamic instability or coagulation profile abnormalities or the fetus is at least at 32 to 34 weeks of gestation at the time of presentation, prompt delivery is undertaken (Table 3-3). If CS delivery is required, red cells, platelets, fresh frozen plasma (FFP), or cryoprecipitate (for hypofibrinogenemia) may be necessary during and after delivery. Although coagulation and platelet abnormalities resolve usually within 48 hours after delivery, thrombocytopenia may continue or become progressive, and thus careful postpartum monitoring is essen-

tial. If persistent, severe postpartum HELLP may require steroids and plasmapheresis. The offspring of patients with both preeclampsia and HELLP also may become thrombocytopenic, although the thrombocytopenia is usually mild. Therapy for HELLP and preeclampsia is directed toward stabilization of the mother, followed by expeditious delivery, after which these disorders usually remit within 3 to 4 days in the majority of patients. HELLP, in particular, occasionally may worsen or even develop postpartum. Prenatal or postnatal corticosteroids have been suggested in several small, randomized studies to hasten resolution of the biochemical abnormalities and thrombocytopenia associated with HELLP, although these studies have not been powered sufficiently to demonstrate an effect on maternal or fetal mortality. One should consider the use of such adjunctive therapies if thrombocytopenia continues to worsen or there is continuing clinical deterioration 5 to 7 days after delivery.

Recommendations. For HELLP, expeditious delivery of the fetus and supportive care of the mother is recommended, including transfusion support through delivery, and corticosteroids and plasma exchange if platelet or coagulation abnormalities persist postpartum.

Table 3-3 Guidelines for management of microangiopathic hemolytic anemias in pregnancy

Scenario	Comments
Preeclampsia, eclampsia For pregnant women considered at risk for preeclampsia, and those with a previous history of preeclampsia, low-dose aspirin is recommended throughout pregnancy, starting with the late first trimester.	For women with a history of pregnancy complications, screening for inherited thrombophilia is not recommended.
Hemolysis, elevated liver function, low platelets (HELLP) For women with HELLP, delivery of the fetus and supportive care of the mother, which may include plasma exchange, are recommended.	
Thrombotic thrombocytopenic purpura (TTP) For women with TTP, delivery of the fetus and supportive care of the mother, including prompt plasma exchange, are recommended.	Consider concurrent corticosteroids
Hemolytic uremic syndrome (HUS) For women with HUS, delivery of the fetus and supportive care of the mother, which may include plasma exchange, are recommended.	Observational studies suggest eculizumab may reduce thrombosis and fetal loss in women with HUS, but it is listed as category C and not recommended in pregnancy but could be used postpartum. The effectiveness of steroids is not established.
Acute fatty liver of pregnancy (AFLP) For women with AFLP, delivery of the fetus and supportive management of the mother are recommended.	Coagulation support is recommended for liver dysfunction and DIC if present, including platelets, fresh frozen plasma (FFP), and cryoprecipitate.
Disseminated intravascular coagulation (DIC) For women with DIC, delivery of the fetus and supportive management of the mother are recommended.	Coagulation support (especially if there is bleeding) is recommended, including platelets, FFP, and cryoprecipitate.
Paroxysmal nocturnal hemoglobinuria (PNH) For women with PNH and past thrombosis or high risk of thrombosis, antepartum and postpartum anticoagulation is recommended (see guidelines, next section).	Observational studies suggest eculizumab may reduce thrombosis and fetal loss in women with PNH but is listed as category C, although benefit outweighs risk.

Adapted from Bates SM et al. *Chest*. 2012;141(2 suppl):e691S–e736S; Woudstra DM, et al. *J Thromb Haemost*. 2012;10:64–72. Corticosteroids for HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome in pregnancy. *Cochrane Database Syst Rev*. 2010;9:CD008148; George JN. *Blood*. 2010;116:4090–4099; Sanchez-Corral P, Melgosa M. *Br J Hematol*. 2010;150:529–542; Fesenmeir MF et al. *Am J Obstet Gynecol*. 2005;192:1416–1419; and Vekemans MC et al. *Blood Coagul Fibrinolysis*. 2015;26:464–466.

Thrombotic thrombocytopenic purpura

The incidence of TTP is increased during pregnancy. It is estimated that 10% to 30% of all adult TTP is obstetric, and 7% of all adult TTP has its onset during pregnancy. TTP may develop in either the second or third trimesters. TTP presenting during weeks 20 to 29 of gestation is associated with severe fetal intrauterine growth restriction, and better outcomes have been observed when TTP presents <20 weeks or >30 weeks. TTP is caused by severe deficiency of ADAMTS13, the von Willebrand factor (VWF)-cleaving protease, which may be congenital (Upshaw-Schulman syndrome) or acquired (autoimmune). The hallmark of TTP is microthrombi in small vasculature, which arise as a direct result of accumulation of large super-adhesive VWF multimers. Microthrombi lead to thrombocytopenia, microangiopathic hemolytic anemia, and neurologic, renal, and central nervous system (hypothalamic) end-organ damage.

Although congenital TTP accounts for only 5% of adult TTP, it accounts for 24% of obstetric TTP. The manifestations of TTP in pregnant and nonpregnant women are similar, and pregnant patients respond equally well to plasma exchange (Table 3-3). A major dilemma in the management of TTP is the difficulty in diagnosis, because overlap with other pregnancy-specific disorders, such as HELLP, may delay diagnosis and lead to increased morbidity and mortality.

Recommendations for acquired TTP. For obstetric TTP, delivery of the fetus and supportive management of the mother is recommended. Plasma exchange is the preferred therapy. Concurrent use of corticosteroids can be considered but is a grade 2C recommendation—understandably, given lack of robust data in this setting. Antiplatelet agents do not appear to be helpful. In women with a history of nonpregnant TTP and subsequently pregnant, wherein the risk of relapse is 15% to 20%, there is no definitive approach to reducing relapse, although options beyond close platelet count monitoring during that subsequent pregnancy include consideration of rituximab prepartum if the ADAMTS13 level prepartum is <20% (though the product monograph recommends to avoid trying to become pregnant for 12 months after receiving rituximab), or serial ADAMTS13 monitoring once pregnant and subsequent initiation of plasma exchange when activity falls to <10%.

Recommendations for congenital TTP. If TTP presents during pregnancy, the likelihood of this being congenital TTP is high (60% in a recent British cohort). Pending results for ADAMTS13, plasma exchange as above is done, then just plasma infusions if the inhibitor screen is negative. The same British group advises for management of subsequent

pregnancies regular plasma infusion (10 mL/kg) from 8 to 10 weeks of gestation every 2 weeks, in combination with LDA. The frequency of infusion is usually increased at 20 weeks of gestation. Delivery is planned at 36 to 38 weeks of gestation. Furthermore, therapy is escalated if the platelet counts drop below 150,000/ μ L at any time.

Hemolytic uremic syndrome

The incidence of HUS also is increased in association with pregnancy. Although some cases of HUS develop near term, the majority of cases develop 3 to 4 weeks postpartum, and their clinical features most closely resemble atypical HUS, with renal failure as the predominant manifestation. The prognosis of postpartum HUS is poor, with persistent renal failure in >25% of affected individuals. Although responses to plasma exchange have been reported, the overall response rate to this intervention is low; nevertheless, a trial of plasma exchange is indicated, particularly given the difficulty in distinguishing TTP and HUS and the potential role of deficiencies of complement regulatory proteins in this syndrome (Table 3-2).

Recommendations. For obstetric HUS, delivery of the fetus and supportive management of the mother is recommended. Treatment in terms of plasma exchange is similar to that for obstetric TTP but if the ADAMTS13 level returns normal, eculizumab should be strongly considered on the presumption that the patient carries a complement mutation “unmasked” by the hormonal state of pregnancy. There is no consensus on the risk of developing recurrent TTP or HUS in subsequent pregnancies; observational studies suggest the risk may be 10% to 20%. Intuitively, that risk would be higher if an underlying complement gene abnormality is identified. The effectiveness of steroids in HUS is not established. Eculizumab should be given for atypical HUS.

Acute fatty liver of pregnancy

AFLP usually occurs in the third trimester and affects primarily primiparas; and although twins are a risk factor, only 1% of cases occur in twins. Symptoms include nausea, vomiting, right upper-quadrant pain, anorexia, jaundice, and cholestatic liver dysfunction. Hypoglycemia is present in >50% of cases. Thrombocytopenia is usually mild, but maternal bleeding is common due to the accompanying coagulopathy resulting from diminished hepatic synthesis of coagulation proteins. Acquired antithrombin deficiency may also occur, and in rare cases could lead to thrombosis. Some cases of AFLP and possibly HELLP may result from fetal mitochondrial fatty acid oxidation disorders, most commonly a deficiency of long-chain 3-hydroxyacyl-coenzyme A dehydrogenase.

Recommendations. For AFLP, delivery of the fetus and supportive management of the mother, and coagulation support for liver dysfunction or DIC, if present, is recommended. If deficiency of ATIII occurs, ATIII concentrate may be given.

Disseminated intravascular coagulation

DIC may occur in the third trimester secondary to PPH from uterine atony or cervical or vaginal lacerations or uterine rupture; amniotic fluid embolism, retained dead fetus, or abruptio placenta. It may also complicate severe preeclampsia/HELLP, AFLP, or puerperal sepsis. DIC is a consumptive coagulopathy characterized by activation of coagulation caused by entrance of thromboplastic or procoagulant substances (eg, amniotic fluid) into the circulatory system. Typically, there is consumption of coagulation factors in both intrinsic and extrinsic pathways, prolonging both the prothrombin time and activated partial thromboplastin time, and consumption of platelets resulting in thrombocytopenia, and presence of breakdown products of fibrin, including fibrin split products and D-dimer. In general, management of DIC is supportive, with platelets, FFP, and cryoprecipitate should severe bleeding occur.

Recommendations. For DIC, delivery of the fetus and supportive management of the mother is recommended; platelets, FFP, and cryoprecipitate may be given to replace platelets and factors consumed. Frequent monitoring of fibrinogen and early replacement of fibrinogen with cryoprecipitate or fibrinogen concentrates are recommended.

Paroxysmal nocturnal hemoglobinuria (PNH)

Paroxysmal nocturnal hemoglobinuria (PNH) is a stem cell disorder usually diagnosed in the early 30s. Thus, although rare, PNH affects females in their childbearing years. PNH is characterized by hypoplastic anemia, bone marrow failure, and hemolysis due to increased susceptibility of red cells to complement-mediated lysis. The defect is a mutated phosphatidyl inositol gene (*PIG-A*), the anchor of the complement regulatory CD55 and CD59 proteins, to the red cell membrane. This defect results in loss of regulation of the terminal complex C5 β -9, leading to red cell lysis. In the case of the red cell, the absence of 2 glycosylphosphatidylinositol (GPI)-linked complement regulatory proteins, CD55 and CD59, increases the sensitivity of red cells to activated complement and complement-mediated lysis. In addition to hemolysis, PNH is characterized by arterial and venous thrombosis that may occur due to depletion of nitric oxide which binds circulating free hemoglobin and may occur at visceral sites, including the inferior vena cava (Budd-Chiari), splenic, hepatic, and cere-

bral veins. Thrombotic risk correlates with expression of GPI-linked proteins on the surface of granulocytes, with the greatest risk associated with a PNH clone >50%.

When PNH occurs in pregnancy, up to 40% end prematurely and only 30% deliver vaginally. Hemolysis leads to smooth muscle dystonia, vasculopathy, and endothelial dysfunction, increasing the risk for premature labor and fetal loss. In the pre-eculizumab era, an 8% to 12% maternal mortality rate was reported in women with PNH, primarily related to postpartum thrombosis, and a 4% to 7% fetal mortality rate. It may be difficult to distinguish thrombotic complications of PNH from thrombotic complications of pregnancy. Because of the high risk of VTE in pregnant women with PNH, at an incidence of 10%, antithrombotic therapy is recommended postpartum for all pregnant patients, and antepartum prophylaxis is indicated for patients with thrombosis-large PNH clones (>50%), prior history of VTE, or recurrent prior late fetal loss. In the past decade, eculizumab, a monoclonal antibody that targets the terminal component of its complement, C5, has greatly improved the treatment of PNH.

Eculizumab is category C, but the benefit of increasing fetal survival and decreasing maternal complications outweighs safety concerns, particularly as major adverse events have not been reported to date, although admittedly the worldwide experience is still not substantial.

Recommendations. For pregnant women with PNH, postpartum prophylactic- or intermediate-dose LMWH is recommended, and in those at high risk for thrombosis (eg, PNH clone >50%, prior VTE), antepartum prophylactic- or intermediate-dose LMWH is recommended. For those patients already on anticoagulation, an intermediate or full therapeutic dose of LMWH would be administered instead of prophylactic-dose LMWH.

Use of eculizumab during pregnancy appears to carry greater benefit than risk. Despite antepartum use of eculizumab, breakthrough hemolysis commonly occurs, necessitating escalation of the dose and frequency of eculizumab.

Bleeding disorders in pregnancy

PPH is a major cause of morbidity and mortality in childbirth. Women with an underlying bleeding disorder are at greater risk for PPH: while several single-center studies have reported PPH in up to a third (most with von Willebrand disease [VWD]), population-based studies indicate lower rates of PPH, about 1.5-fold greater than women without a bleeding disorder. A summary of the management of bleeding disorders in pregnancy, including preferred agents, target levels, and dosing, is found in Table 3-4.

Table 3-4 Specific factor replacement in inherited bleeding disorders peripartum

Factor deficiency	Patients' factor level (normal)	Desired level	Recommendation
VWD type 1	<50%	>100%	VWF concentrate 40–60 IU/kg, then 20–40 IU/kg q 12 h, then daily 3–5 days if vaginal delivery, 5–7 days if cesarean
VWD types 2, 3	<50%	>100%	VWF concentrate 60–80 IU/kg, then 40–60 IU/kg q 12 h, then daily 3–5 days if vaginal delivery, 5–7 days if cesarean
FI (fibrinogen)	<0.5 g/L	1–1.5 g/L × 3 days	Pregnancy prophylaxis: fibrinogen concentrate 50–100 mg/kg twice a week to maintain level at >1 g/L (more during labor) × 3 days. Cryoprecipitate 15–20 mL/kg, SD-FFP 15–30 mL/kg, TXA 15–20 mg/kg IV, then 1 g po tid.
FII	<20% (50%–150%)	20%–40%	PCC 20–40 U/kg, then PCC 10–20 IU/kg q 48 h to maintain levels for at least 3 days
FV	<20% (50%–150%)	20%–40%	FFP 15–20 mL/kg, later FFP 10 mL/kg q 12 h for at least 3 days. For severe bleeding or cesarean, give platelet transfusion (FV+VIII give DDAVP, FFP).
FVII	<20% (50%–150%)	>40%	rFVIIa 15–30 µg/kg q 4–6 h for at least 3–5 days
FVIII, FIX	<50% (50%–150%)	>100%	FVIII carrier: FVIII concentrate 20–40 IU/kg; FIX carrier: 40–50 IU/kg
FX	<30% (50%–150%)	>40%	PDFX concentrate 1500 U (18.8–25 U/kg), PCC 10–20 U/kg qd × 3 days, FFP
FXI	<15%–20% (70%–150%)	>30%–40%	If bleeding phenotype or prior h/o PPH-FXI concentrate 15–20 U/kg if available; FFP, TXA alone at 1 g qtg. rFVIIa for inhibitors
FXIII	<30% (70%–150%)	>20%	Pd-FXIII 20–40 U/kg × 1, rFXIII-A 35 U/kg, cryoprecipitate, FFP

Adapted from Pavord S et al, *BJOG* 2017;124:e193–e263. It should be recognized that these represent expert opinion recommendations, and treatment duration and intensity are based on not only the factor level but historical assessment of the bleeding phenotype.

DDAVP, 1-desamino-8D-arginine vasopressin; FFP, fresh frozen plasma; PCC, prothrombin complex concentrate; PDFX, plasma-derived FX; Pd-FXIII, plasma-derived FXIII; PPH-FXI, postpartum hemorrhage-FXI concentrate; rFXIII-A, recombinant FXIII; SD-FFP, solvent detergent fresh frozen plasma; TXA, tranexamic acid; VWD, von Willebrand disease; VWF, von Willebrand factor.

PPH typically occurs due to a failure of the uterus to contract after delivery. Primary PPH is defined as an estimated blood loss of >500 mL at the time of vaginal delivery, or >1,000 mL at the time of a cesarean delivery, and affects 4% to 6% of all pregnancies. Secondary PPH is excessive vaginal bleeding occurring between 24 hours and 6 weeks after childbirth. The most common causes of PPH in the general obstetric population, besides uterine atony, are retained placenta/products of conception, and genital tract trauma. Table 3-5 reviews the multifactorial nature of PPH.

Women with inherited bleeding disorders can have these same risk factors, as well as the additional risk factor of their coagulation defect. In the general population, most PPHs are primary. In women with bleeding disorders, delayed (or secondary) PPH is much more common and has been reported in 20% to 25% of women with VWD, 2% to 11% of hemophilia carriers, and 24% of women with factor XI deficiency. Risk factors for uterine atony include prolonged induced or augmented labor and expectant rather than active management of the third stage of labor. Therefore, in women with inherited bleeding disorders,

these factors should be minimized to reduce the risk of PPH. Active management of the third stage of labor may include the administration of prophylactic uterotronics (single or double) to increase muscle contractility and controlled traction of the umbilical cord during the delivery of the placenta. Hemostatic management also may reduce the risk of PPH. Factor levels should be assessed in the third trimester of pregnancy, and prophylactic factor replacement given at delivery to those with subtherapeutic levels (Table 3-4). Finally, care must be taken to minimize genital and perineal trauma to reduce the risks of both PPH and perineal hematomas. Perineal (or vulvar) hematomas, a rare complication of vaginal birth, occur with some frequency in women with bleeding disorders and contribute to the increased incidence of PPH. In one patient series, the prevalence of perineal hematoma was much higher in women with inherited bleeding disorders (1% to 6%) as compared with a reported 0.2% in the general population. Even after discharge from the hospital, women with inherited bleeding disorders require close follow-up during the postpartum period. In the general obstetric population, the median duration of bleeding after delivery is 21 to 27 days.

Table 3-5 Multifactorial nature of postpartum hemorrhage

Sociodemographic	Asian ethnicity
	Hispanic ethnicity
	Age > 30 y
Obstetric	Prolonged stage 3 labor
	Preeclampsia
	Retained placenta
	Abnormal placentation
	Previous PPH
	Placental abruption
	Multiple gestation
	Fetal macrosomia
	HELLP syndrome
	Polyhydramnios
	Intrapartum oxytocin exposure
	Induction of labor
	Prolonged labor
Surgical	Emergency cesarean delivery
	Elective cesarean delivery
	Forceps delivery
	Vacuum delivery
	Episiotomy
	Perineal suture
Medical/systemic	Antepartum hemorrhage
	von Willebrand disease, coagulopathies, platelet disorders
	Anemia < 9 g/dL
	Pyrexia in labor
	Obesity: BMI > 35 kg/m ²
	Cardiac disease

Modified from Table 3 of Abdul-Kadir R et al, *Transfusion*. 2014;54(7):1756–1768. Courtesy of Sweta Gupta.

HELLP, hemolysis, elevated liver function tests, low platelets; PPH, postpartum hemorrhage.

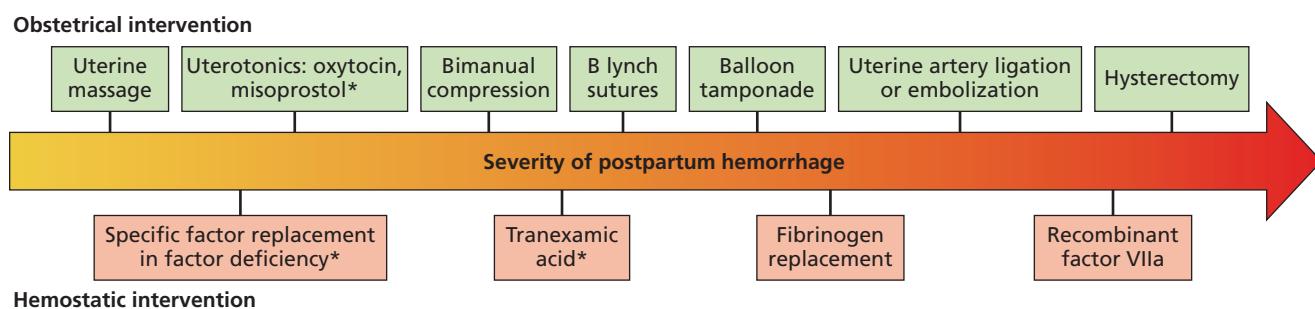
A case-control study revealed that women with inherited bleeding disorders have significantly longer postpartum bleeding than controls, even when they receive appropriate hemostatic treatment. In VWD, the pregnancy-induced increase in coagulation factor levels starts to decline 3 to 7 days after delivery and return to prepregnancy levels within 7 to 21 days of delivery. Therefore, close postpartum monitoring of women with bleeding disorders is recommended for, at minimum, 3 weeks and up to 6 weeks. Figure 3-2 depicts the continuum of obstetrical and hematological interventions that are available peripartum in

the bleeding disorder patient. Figure 3-3 depicts the general approach to the prevention and treatment of PPH in women with an underlying bleeding disorder.

Von Willebrand disease

VWD is the most common inherited bleeding disorder. Although approximately 1% of the general population is affected, only 0.1% are symptomatic, but many are unaware of their diagnosis. Clinically, the disease is characterized by mucosal bleeding, including menorrhagia, bruising, epistaxis, and postoperative bleeding. There are several variants. Type 1 VWD is a partial, quantitative deficiency of VWF, and accounts for 70% to 75% of all VWD cases. Type 2 VWD, accounting for 20 to 25% of the disease, consists of 4 subtypes: type 2A is caused by a qualitative defect in VWF in which high-molecular-weight VWF (high-molecular-weight multimers) are reduced, resulting in a more severe phenotype; type 2B is characterized by a gain-of-function mutation resulting in increased affinity and binding of VWF to platelet GP1b, resulting in thrombocytopenia and spontaneous platelet aggregation; type 2M is characterized by decreased affinity of VWF for its platelet receptor glycoprotein 1b (GPIb); and type 2N is characterized by a loss-of-function mutation in which the VWF binding site for factor VIII (FVIII) is mutated, resulting in greatly reduced FVIII, which may be confused with mild hemophilia A. Type 3 VWD accounts for <5% of the disease and is characterized by a severe deficiency in VWF, resulting in a corresponding deficiency of FVIII.

Under the regulation of estrogen that occurs in pregnancy, the levels of VWF, factor FVIII, and most other clotting factors increase, although not as high as the increase seen physiologically in a normal pregnancy, so likely explaining the increased risk of PPH and evolving consensus to replace to a level >~100% as opposed to historically ~50%. In general, the rise begins in the early second trimester and peaks between 29 and 35 weeks. For this reason, a diagnosis of VWD may be masked if VWF levels are performed when a patient is pregnant, particularly within 6 to 8 weeks of delivery. Thus, whenever possible, the preconception VWF level and bleeding history should be established. During pregnancy, most patients with type 1 VWD normalize their levels of VWF and FVIII, although those with more severe disease may not. Given the somewhat unpredictable nature of these responses, measurement of VWF levels should be performed around 34 to 36 weeks; levels generally remain fairly stable through the remainder of pregnancy, and thus levels obtained at this time allow for a delivery plan to be developed. Although levels of VWF may increase in patients with type 2 VWD, functional levels may not be significantly enhanced due

**Figure 3-2**

The continuum of obstetrical and hemostatic interventions in the prevention and treatment of PPH. The asterisk denotes consideration in prevention of PPH if underlying bleeding disorder and/or placental previa, twin gestation, or antepartum hemorrhage. Redrawn from Kouides PA, *Blood Adv*. 2017;1(11):699–702.

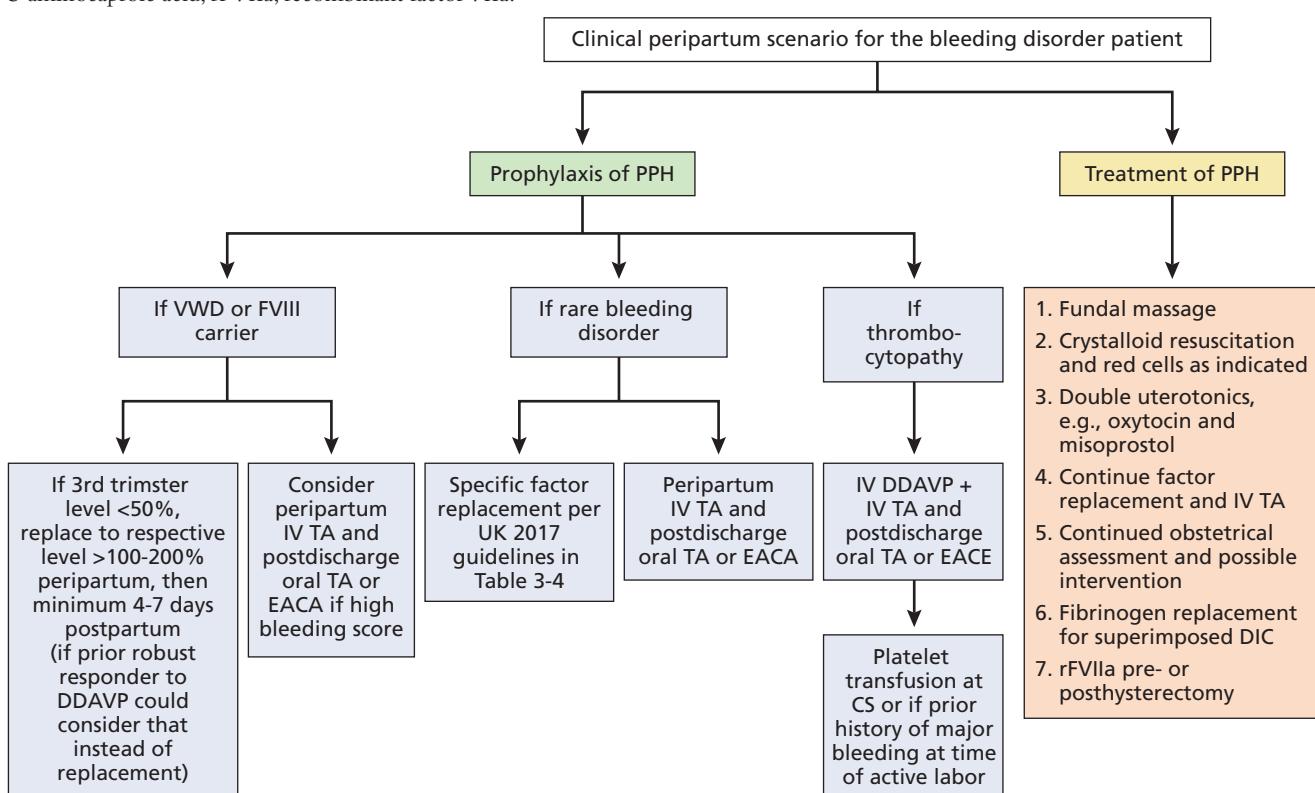
to the production of a functionally defective protein. Levels of VWF generally do not increase during pregnancy in patients with type 3 VWD.

In most cases, the physiologic increase in VWF during pregnancy exceeds the minimum 50-IU/dL VWF level recommended for epidural anesthesia in type 1 VWD. Thereafter, it is generally judicious to remove the catheter as soon as possible after delivery is completed. Postpartum, the decline in VWF levels generally occurs over 2 to 3

weeks, and it may be unpredictable and occasionally precipitous; thus, the period of 3 to 6 weeks postpartum is considered a particularly vulnerable time for postpartum bleeding and close follow-up is recommended. Not only is postpartum bleeding more common in pregnant women with VWD, so too is transfusion requirement, longer hospital length of stay, and mortality, which may be up to 1.2%. Several therapeutic options are available. Desmopressin (1-desamino-8D-arginine vasopressin [DDAVP])

Figure 3-3

Prevention and management of PPH in patients with bleeding disorders. CS, cesarean section; EACA, ϵ -aminocaproic acid; rFVIIa, recombinant factor VIIa.



is an option if the patient has been documented to be an excellent responder in the past but must be used very cautiously due to the risk of hyponatremia in the setting of often vigorous fluid replacement with hypotonic fluids. Consequently, as another option for patients with type 1 VWD, including those allergic or unresponsive to desmopressin, and those with type 2 and type 3 VWD, VWF concentrate is recommended and continued for up to 3 to 7 days postpartum, as required by disease severity and mode of delivery (Table 3-4).

Recommendations. Based on case reports and expert opinion, it is recommended that pregnant women with type 1 VWD and VWF levels <50 IU/dL in the eighth month of pregnancy, and those with type 2 or 3 VWD, receive VWF concentrate at the time of active labor up to 3 to 7 days postpartum. Central neuraxial anesthesia is safe in type 1 VWD after achieving a VWF level >50%. But regarding types 2 and 3 VWD, the 2017 Royal College of Obstetricians and Gynaecologists guidelines advise "that neuraxial anesthesia be avoided unless VWF activity is more than 50% and the haemostatic defect has been corrected; this may be difficult to achieve in type 2 and central neuraxial anesthesia should not be given in cases of type 3." In type 2N, central neuraxial anesthesia is safe if the FVIII level is replaced to >50%.

Monitoring for postpartum bleeding is recommended for at least 3 weeks and preferably 6 weeks postpartum. VWF concentrate is preferred over DDAVP at delivery. This is because women commonly receive 1 to 2 liters of fluid at the time of vaginal delivery and 2 to 3 liters at the time of CS, and desmopressin may result in fluid retention, life-threatening hyponatremia, and/or seizures. However, in an excellent DDAVP responder and in a controlled setting where fluids can be carefully monitored, both the 2017 United Kingdom and National Hemophilia Foundation guidelines allow for DDAVP use—albeit with caution.

Regarding replacement therapy, historically the target level was 50% or higher but recent studies strongly suggest undertreatment resulting in increased blood loss, so postpartum replacement ideally should aim for VWF levels >~100 IU/dL (ie, closer to levels that are observed in normal pregnant women). It is possible that the undertreatment is in part also because the dosing is not weight based, that is, taking into consideration the increased plasma volume peripartum.

Regarding adjunctive use of antifibrinolytic therapy, in considering the risk/benefit, it would seem reasonable to use TXA, 1 gram IV load at delivery in the type 2 or 3 VWD patient and the "severe" type 1 patient who has not normalized their levels in the third trimester. TXA can be considered thereafter in these patients at 1 gram orally 3 times a day for 7 to 21 days postpartum, in tracking post-

partum flow in terms of changing frequency of sanitary napkins <2 hours; just as the clinician would intervene if menstrual flow was this frequent during menstruation. In the type 1 patient who has normalized their VWF levels, expectant management without prophylactic antifibrinolytic therapy is reasonable postpartum unless they undergo a CS or have a prior history of PPH or an increased bleeding score of >10. Monitoring for bleeding is recommended for at least 3 weeks, as noted above.

Hemophilia carriers

Postpartum bleeding may occur in 10% of hemophilia carriers and may lead to significant blood loss and anemia, in some cases requiring transfusion. Interestingly, the factor level does not predict bleeding risk: up to 30% of hemophilia carriers, even with normal factor VIII and IX levels, may have high bleeding scores; and up to 30% may be considered to be mild hemophilia with contributing factors for low levels including the type of mutation, the degree of skewed X chromosome (extreme Lyonization) and concurrent VWF level, which in turn can be influenced by the ABO type.

In the hemophilia carrier expecting an affected infant, the risk of intracranial hemorrhage is 2.5% compared to 0.06% in the general population (odds ratio 44-fold) and the risk of extracranial hemorrhage is 3.7% compared with 0.47% (odds ratio 8-fold). The majority of cases were due to instrumentation (vacuum extraction or forceps). Nonetheless, although not proven conclusively due to lack of randomized data, CS delivery is recommended over vaginal delivery to reduce that risk. In high-risk infants, the critical issue is the availability of a multidisciplinary team in an obstetric unit with facilities for high-risk deliveries. Of course, the problem is that many carriers are not diagnosed until *after* delivery, and even in those who are known carriers, an affected infant may not be anticipated if they are not properly counseled. It should be recognized that preconception counseling with genotyping is currently available, as well as pre- and postimplantation options, including preimplantation genetic diagnosis and postimplantation fetal DNA sex determination, chorionic villus sampling, and amniocentesis.

Recommendations: For hemophilia A (or B) carriers with FVIII (or factor IX [FIX]) levels <50 IU/dL or severe past bleeding history, recombinant FVIII (or FIX) concentrate is recommended at the time of neuraxial anesthesia and continued for up to 3 to 7 days postpartum, ideally aiming for a target factor level of >100 IU/dL. In women with hemophilia in whom an affected infant is anticipated, because of the potential risk of central nervous system bleeding, CS delivery should be offered. Vacuum extrac-

tion and forceps should be avoided because of the risk of cephalohematoma and intracranial hemorrhage. External cephalic inversion should be avoided. A team approach (Table 3-1), including the obstetrician, anesthesiologist, and hematologist, and communication regarding mode of delivery and factor replacement, is critical in managing carriers. FVIII concentrate is preferred over the use of desmopressin for delivery. Although mild hemophilia A carriers may prefer desmopressin for treatment of minor procedures akin to its use in VWD patients, as noted above, is discouraged at delivery due to the risk of hyponatremia; though as in the VWD patient, if known to be an excellent DDAVP responder and fluids are carefully monitored, then DDAVP can be used.

Rare bleeding disorders

The rare bleeding disorders include inherited deficiencies of coagulation factors I, II, V, VII, X, XI, and XIII, which represent 5% of all inherited bleeding disorders. There is a wide spectrum of bleeding severity, from none to severe, and it is difficult to predict bleeding risk. Thus, a diagnosis of a rare bleeding disorder may not come to clinical attention until a woman, even with prior bleeding history, experiences postpartum bleeding. In general, risk is related to factor levels, but not entirely. The key to optimal delivery management is awareness of the diagnosis, testing the appropriate factor level at the eighth month of pregnancy, and replacement therapy at delivery for factor deficiency. Because coagulation factors generally increase during pregnancy, a diagnosis may be masked and testing should precede pregnancy whenever possible. In particular, factors I, VII, VIII, VWF, X, XII, and XIII increase during pregnancy, whereas factors II, V, IX, and XI show minimal or no increase. In general, fibrinogen levels of >1.0 to 1.5 g/L, FII >20 to 40 IU/dL, FV >20 to 40 IU/dL, FVII >40 IU/dL, FX >40 IU/dL, FXI >30 to 40 IU/dL, and FXIII >20 IU/dL are recommended, respectively, for each deficiency state, at the time of delivery (Table 3-4). When possible, preconception counseling should be provided and genetics and reproductive choices should be discussed. Although prenatal diagnosis with chorionic villus sampling and amniocentesis is possible, few obtain it, given the associated 1% to 2% fetal loss. As noted, a team approach with a coordinated management plan optimizes outcomes for affected women.

Recommendations. For an affected woman or a known asymptomatic heterozygous carrier, consanguinity should be established, and if so, CS delivery should be offered to reduce the risk of intracerebral hemorrhage. In general, central neuraxial anesthesia should be avoided unless replacement can adequately restore hemostasis fully.

Based on expert opinion, for rare bleeding factor deficiency states, FFP or factor concentrate to bring factors to hemostatic levels (Table 3-4) is recommended at the time of active labor and for 3 to 4 days postpartum for vaginal delivery and up to 5 to 7 days for cesarean delivery. Adjunctive treatment with TXA 1 g po tid should be considered at delivery based on that patient's historical bleeding phenotype.

Hypofibrinogenemia

Fibrinogen abnormalities, including afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia may be associated with hemorrhagic and thrombotic pregnancy complications, including PPH, spontaneous abortion, and placental abruption. Up to 30% of patients with congenital fibrinogen deficiency have thrombotic complications, most commonly first-trimester abortion; this is common in those with afibrinogenemia, but less frequent in those with hypofibrinogenemia or dysfibrinogenemia. Fibrinogen plays an important role in placental implantation and maintenance of placental competency during pregnancy. When defects in fibrinogen conversion to fibrin occur during pregnancy—whether from deficient or defective fibrinogen—placental separation, miscarriage, spontaneous abortion, and hemorrhage may occur. The high rate of pregnancy complications may be reduced by fibrinogen replacement (Table 3-4). Several experts suggest that fibrinogen replacement should be initiated as early as possible in pregnancy.

Recommendations. For pregnant women with fibrinogen <0.5 g/L, prophylaxis throughout pregnancy with fibrinogen concentrate, initially 50 to 100 mg/kg twice weekly adjusted to maintain fibrinogen activity >1 g/L to achieve a level of 1.5 g/L during labor and for 3 days postpartum. For pregnant women with thrombotic dysfibrinogenemia, afibrinogenemia, or hypofibrinogenemia and other risk factors for VTE, thromboprophylaxis should be considered.

Factor XIII deficiency

Factor XIII deficiency is a rare disorder, occurring in 1 in 2 million people, and is associated with pregnancy loss in over 90% of cases. Long-term prophylaxis is advised in all factor XIII-deficient patients with a personal or family history of bleeding and those with FXIII activity <0.1 IU/mL.

Recommendations. For pregnant women with factor XIII deficiency, it is recommended that the frequency of prophylaxis be increased from every 4 weeks to 10 to 40 IU/kg every 2 to 3 weeks, aiming for a FXIII activity above 20 IU/dL. At the time of delivery, an additional dose of 10 to 40 IU/kg is advised.

Thromboembolism and thrombophilia in pregnancy

In general, the hemostatic system is in a perfect balance of anticoagulant factors and procoagulant factors, lest the patient bleeds or clots excessively. During pregnancy, hormonal changes, specifically increases in estrogen and progesterone, lead to a transient hypercoagulable state. This transient procoagulant state was teleologically important, most likely to protect against fatal hemorrhage at birth or with miscarriage. Factor levels that increase during pregnancy include factors I (fibrinogen), VII, VIII, X, VWF, and plasminogen activator inhibitor (PAI-1 and PAI-2), all of which return to normal beginning 2 to 3 weeks postpartum. In parallel, a substantial decrease in free protein S levels occurs because of increased levels of C4b-binding protein. In order to avoid misdiagnosis of protein S deficiency, it is important to recognize that protein S levels are as low as 30% in the second trimester and 26% in the third trimester of normal pregnancies. An increase in activated protein C resistance in the absence of factor V Leiden (FVL), unexplained by the decrease in free protein S, also is observed in some pregnant patients, particularly in the third trimester. Furthermore, there also is a decrease in tissue plasminogen activator activity. Besides these prothrombotic changes, there are numerous acquired prothrombotic risks that arise in pregnancy, including progressive venous obstruction from the enlarging uterus or relative immobility as the pregnancy progresses (particularly if bedrest is prescribed); or if varicose veins develop or the pregnant patient is postoperative, wherein finally the hemostatic balance is tipped, resulting in thrombosis. As such, the hypercoagulable state of pregnancy is almost always a multifactorial process.

The end result of this transient hypercoagulable state is a 5- to 10-fold increased risk of VTE. In absolute terms, the risk of VTE is approximately 1:1,000 (0.66 to 2.22 of 1,000 pregnancies) compared to 1:10,000 in an age-matched, nonpregnant female not on oral contraceptives. This increased absolute risk also is associated with increased mortality. In developing countries, VTE is a leading cause of death. There is an approximately 5- to 10-fold increased risk for VTE in the antepartum period and a 22- to 84-fold increased risk in the postpartum period.

While the risk in the postpartum period appears to be the highest, probably due to pronounced vascular congestion and continued changes in the hemostatic factors, the practitioner should be aware that risk is present even in the first trimester before anatomical changes ensue. Another little-known fact is that while the majority of VTEs are DVTs and 20% are pulmonary emboli (PE), the risk for PE is far greater in the postpartum period; and while the risk for VTE is greatest for the first 6 weeks postpartum, it

persists up to 12 weeks. Not surprisingly, the risk is 4- to 5-fold greater after an emergency cesarean section when compared to a vaginal delivery (a planned CS is minimal increased risk compared to a vaginal delivery). Also, it should be pointed out that there is a concurrent risk of arterial thromboembolism 3- to 4-fold in pregnant women. Figure 3-4 depicts the odds ratios for various noninherited prepartum, antepartum, and postpartum risks. In patients with inherited thrombophilia, these additional risks should also be considered in considering antepartum and/or postpartum thromboprophylaxis.

Pregnancy-associated DVT is more often proximal and massive than in the nonpregnant setting and usually occurs in the left lower extremity. In contrast, distal DVT occurs with similar frequency in the left and right lower extremities. The left-sided and proximal over distal vein predominance of VTE may reflect compression by the gravid uterus of the left iliac vein as it crosses the right iliac artery and lumbar vertebrae.

Regarding superimposed genetic thrombophilia, between 20% and 50% of all thromboembolic events that occur in pregnant women are associated with a thrombophilic disorder. The absolute risk for the various genetic thrombophilias is outlined in Table 3-6.

Given the differing absolute risks of thrombosis, thromboprophylaxis should be individualized based on the type of thrombophilia, presence of homozygous or heterozygous mutations, history of past VTE or pregnancy complications, and presence or absence of a family history of VTE, as well as the presence of additional prothrombotic conditions (outlined in Figure 3-4). The cumulative VTE risk then must be weighed against the bleeding risk with LWMH estimated at the upper limit of ~3%.

Diagnosis of VTE in pregnancy

A diagnosis of VTE often is difficult in pregnancy because of a lack of validation of standard diagnostic studies in this population. Although an abnormal compression ultrasound (CUS) is considered sufficient for diagnosis of DVT during pregnancy, a normal CUS does not reliably exclude DVT because of the low sensitivity for isolated iliac DVT; magnetic resonance imaging (MRI) is the “gold standard” test of choice to diagnose iliac DVT, though an alternative strategy is negative serial CUS with imaging of the iliac veins. Chest radiography (CXR) is recommended by the American Thoracic Society as the first-line, radiation-associated procedure for diagnosis of PE unless DVT signs/symptoms are present; then a CUS should be performed. Ventilation perfusion scan (V/Q) is generally preferred if the CXR is normal. Computed tomographic pulmonary angiography (CTA) is generally preferred

Table 3-6 Absolute VTE risk in pregnancy and postpartum in asymptomatic women with inherited thrombophilia with and without a family history

Inherited thrombophilia	Family history of VTE*	Combined antepartum and postpartum risk (%)	95% CI
FVL			
Heterozygous	No	1.2	0.8–1.8
Heterozygous	Yes	3.1	2.1–4.6
Homozygous	No	4.8	1.4–16.8
Homozygous	Yes	14.0	6.3–25.8
PGM			
Heterozygous	No	1.0	0.3–2.6
Heterozygous	Yes	2.6	0.9–5.6
Homozygous	No	3.7	0.2–78.3
Homozygous	Yes	—	
Compound FVL/PGM†		5.5	0–21.92
PC deficiency	No	0.7	0.3–1.5
	Yes	1.7	0.4–8.9
		0	0–25.9 (total)
		0	0–79.4 (no prophylaxis)
PS deficiency	No	0.5	0.2–1.0
	Yes	6.6	2.2–14.7
		0	0–32.4 (total)
		0	0–48.9 (no prophylaxis)
AT deficiency	No	0.7	0.2–2.4
	Yes	3.0	0.08–15.8
		8.3	1.4–35.4 (total)
		14.3	2.6–51.3 (no prophylaxis)

Reprinted from Skeith L. *Hematology Am Soc Hematol Educ Program*. 2017;2017:160–167, and adapted from Bates SM et al. *J Thromb Thrombolysis*. 2016;41(1):92–128, with calculated risk based on a baseline VTE incidence of 1.4 per 1000 pregnancies from a non-family-based population study [Kane EV et al, *Eur J Obstet Gynecol Reprod Biol*. 2013;169(2):223–229]. The antepartum and postpartum risks are roughly equal (half the total events occurring antepartum and half postpartum). Certain thrombophilias such as heterozygous FVL, heterozygous PGM, and PS deficiency have a higher VTE risk reported in the postpartum period.

*The definition of family history varies according to each study.

†Based on data from Gerhardt A et al. *Blood*. 2016;128(19):2343–2349, which includes a population with and without family history of VTE.

AT, antithrombin; FVL, factor V Leiden; PC, protein C; PGM, prothrombin gene mutation; PS, protein S;VTE, venous thromboembolism.

if the screening CXR is abnormal or the V/Q scan not available. Fetal radiation exposure is less with CTA than V/Q, while maternal radiation exposure is higher with CTA than V/Q, resulting in a slight increase in long-term maternal breast and lung cancer. Regarding fetal risk overall, the combined exposure of all 3 modalities carries an estimated fetal radiation exposure less than 0.5 mSv. This exposure is 100 to 200 times less than the dose felt to be associated with a significant risk of fetal anomalies. Any discussion of these real but low risks of long-term radiation exposure should be in the context of the mortality of an untreated PE of 20% to 30%. As D-dimer is elevated

in pregnancy, it should not be used to exclude PE in pregnant women.

Treatment of pregnancy-related VTE

The anticoagulant of choice in pregnancy is LMWH (Table 3-7). LMWH is preferred over vitamin K antagonists (VKAs; eg, warfarin) as well as over subcutaneous unfractionated heparin (SUH). LMWH is associated with less bleeding risk, more predictable therapeutic response, lower risk of heparin-induced thrombocytopenia (HIT), longer half-life, less bone density loss, and less local skin hemorrhage. LMWH (as well as SUH) does not cross the

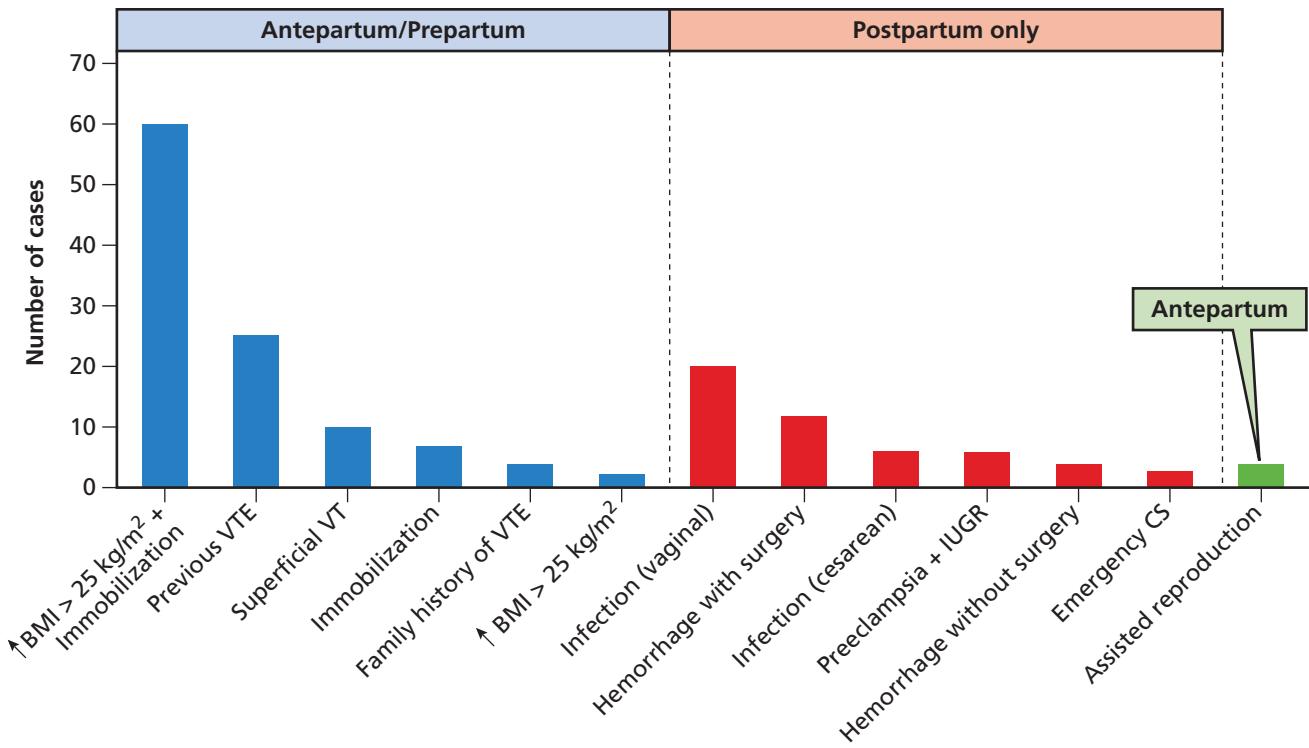


Figure 3-4 Odds ratios of prepartum, antepartum, and postpartum risks (notwithstanding specific genetic thrombophilia). CS, cesarean section; IUGR, intrauterine growth restriction; VT, vein thrombosis. Adapted from Bourjeily G et al, *Lancet*. 2010;375(9713):500–512.

placenta, and numerous studies have confirmed its use is safe for the fetus. Warfarin is embryopathic and fetotoxic. However, as discussed in the section on mechanical heart valves, in select cases, warfarin may be used antepartum when the risk of maternal thromboembolism clearly outweighs these other risks. Presently, the direct oral anticoagulants should not be used antepartum based on animal studies showing increased reproductive risk and insufficient data in humans to date.

Recommendations at time of delivery. Full-dose LMWH should be discontinued at least 24 hours, and prophylactic dose LMWH should be discontinued at least 12 hours before the induction of labor or CS delivery (or expected time of neuraxial anesthesia), given the concern for epidural hemorrhage at the time of an epidural and postpartum hemorrhage (Table 3-8). Although randomized clinical trial evidence is lacking, most experts would advise a planned induction if VTE develops within 8 weeks before delivery. In that time frame, hospital admission to switch to intravenous SUH (as would be done for a patient with an artificial valve) would also be appropriate if the VTE occurs within 4 weeks before delivery. If the VTE occurs within 2 weeks before delivery, a retrievable filter should be considered and/or if a CS is being planned.

Alternatively, the patient can be switched to SUH 2 to 5 weeks before the delivery date to allow for the option of an epidural, particularly in case of premature delivery, although a prolonged anticoagulant effect has been observed up to 28 hours with every 12-hourly dosing. LMWH or SUH can be resumed within 4 to 6 hours after a vaginal delivery and 6 to 12 hours after a CS delivery, or longer if peripartum bleeding occurs. In the event that SUH or LMWH is held, sequential pneumatic compression devices should be used. If an epidural is in place, prophylactic LMWH should be resumed no sooner than 4 hours after epidural removal, and full dose LMWH no sooner than 24 hours after epidural removal.

Recommendations. For pregnant women with acute VTE, adjusted, full-dose subcutaneous LMWH at the standard dose of enoxaparin 1 mg/kg twice a day is recommended during pregnancy and continued or transitioned to a VKA targeted to an international normalized ratio (INR) of 2.0 to 3.0 for at least 6 weeks postpartum, for a minimum total duration of 3 months. If INR monitoring cannot logically be done, then remaining on LMWH is an option. VKAs are permissible during breastfeeding and should be started the evening after delivery. In patients markedly prothrombotic with multiple risk factors for VTE in the prior

Table 3-7 Features of anticoagulants used or contraindicated in pregnancy

Agent	Pros	Cons
Danaparoid	Can be used in HIT Does not appear to cross the placenta	Injection Not available in the United States
Direct oral anticoagulants	Theoretically may have a role postpartum for thromboprophylaxis in nonbreastfeeding patients pending ongoing studies and local availability of an antidote	Cannot be used in pregnancy as they cross the placenta Cannot be used if breastfeeding
Fondaparinux	Can be used in HIT Once daily dosing	Injection Does cross the placenta Cannot be used if breastfeeding
Low-molecular-weight heparin (LMWH)	Does not cross the placenta Most bioavailable of agents, allowing daily to BID dosing Lower rate of HIT than UFH Can be used if breastfeeding	Injection Costlier than UFH
Unfractionated heparin (UFH)	Does not cross the placenta Can be used if breastfeeding	Injection Moderate risk of osteoporosis with use through pregnancy Risk of HIT
Warfarin	Oral use postpartum is advantageous Can be used if breastfeeding	Does cross the placenta with 4%–10% risk of embryopathy and increased fetal loss and neurodevelopmental abnormalities

HIT, heparin-induced thrombocytopenia.

Table 3-8 Neuraxial anesthesia recommendations: timing of neuraxial anesthesia in relation to pharmacologic prophylaxis

Stage	Anticoagulation
Antepartum or intrapartum	
UFH prophylaxis ($\leq 10,000$ IU/d)	No contraindication to timing of heparin dose and performance of neuraxial blockade Note: the American Society for Regional Anesthesia and Pain Medicine advises waiting 4–6 h after last prophylactic UFH dose
UFH therapeutic	Wait 6 h after last dose before neuraxial blockade or PTT
LMWH prophylaxis	Wait 12 h after last dose before neuraxial blockade
LMWH therapeutic	Wait 24 h after last dose before neuraxial blockade
Postpartum	
UFH prophylaxis ($\leq 10,000$ IU/d)	No restriction on epidural catheter removal or spinal needle placement
UFH therapeutic	Wait at least 1 h after epidural catheter removal or spinal needle placement
LMWH prophylaxis	Wait at least 4 h after epidural catheter removal or spinal needle placement
LMWH therapeutic	Avoid therapeutic placement with epidural catheter in situ; wait at least 24 h after catheter removal or spinal needle

Reproduced with a minor modification from D'Alton ME et al, *Obstet Gynecol*. 2016;128(4):688–698, with permission.

LMWH, low-molecular-weight heparin; PTT, partial thromboplastin time; UFH, unfractionated heparin.

6 months, anticoagulation may be considered for up to 12 weeks postpartum.

In general, the odds ratio for VTE is 10.8 for up to 6 weeks postpartum as compared with the same period 1 year later, while the odds ratio is 2.2 for VTE from 7 to 12 weeks postpartum compared with the same period 1 year later. While studies do not conclusively support routine Xa monitoring in pregnant women requiring full-dose anticoagulation, periodic Xa monitoring is reasonable in the obese patient, since Xa levels fall by 25% in the third trimester as the blood volume rises. Monitoring is also reasonable if renal function is reduced. Pharmacokinetics is optimized if dosing for an acute VTE is at 1 mg/kg twice daily of enoxaparin with an anti-Xa target of 0.6 to 1.0 IU/mL, or at 1.5 mg/kg/d once-daily dosing, with an anti-Xa target 0.8 to 1.6 IU/mL, after hospital discharge. For pregnant women with hemodynamically unstable PE, tissue plasminogen activator can be given if the benefit outweighs the risk—a reported 6% to 15% fetal death rate and 8% to 30% major maternal bleeding rate.

Prophylaxis for women at risk for pregnancy-related VTE

The reader is cautioned that risk-based prophylaxis is an area of uncertainty and controversy. Besides genetic thrombophilia, one must take into consideration the numerous prepartum, antepartum and postpartum risk factors depicted in Figure 3-4, such as maternal body mass index (particularly coupled with immobilization), CS delivery, or PPH requiring transfusion.

Recommendations regarding antepartum prophylaxis. For women with multiple prior VTE, prior VTE with high-risk thrombophilia, or prior VTE with acquired thrombophilia, intermediate to treatment dose LMWH or unfractionated heparin (UFH) is recommended. Women with idiopathic prior VTE, prior VTE with pregnancy or oral contraceptives, prior VTE with low-risk genetic thrombophilia, or high-risk thrombophilia require prophylactic dose LMWH or UFH.

For pregnant women with low-risk asymptomatic genetic thrombophilia, prior provoked VTE from trauma or postoperative state or low-risk thrombophilia regardless of a family history of VTE, antepartum clinical vigilance is recommended as the threshold is not “crossed” of the VTE risk exceeding bleeding risk on LMWH. However, antepartum prophylaxis can be considered for pregnant women with no prior VTE and known moderate-to-severe ATIII deficiency but positive family history. Women with ATIII deficiency also may be candidates for antithrombin concentrates peripartum.

Recommendations regarding postpartum prophylaxis. Women with multiple priorVTE, priorVTE with high-risk thrombophilia, or priorVTE with acquired thrombophilia, 6-week treatment dose LMWH or VKA targeted to INR 2.0 to 3.0 is recommended.

For women with idiopathic priorVTE, priorVTE with pregnancy or oral contraceptives, priorVTE with low-risk thrombophilia, high-risk thrombophilia with or without family history, or prior provoked VTE require 6 weeks of anticoagulation, as noted, either with VKA as above or an LMWH at low or intermediate dose. A retrospective case series suggests low-dose LMWH postpartum prophylaxis may be inadequate compared to intermediate dose LWMH, and a randomized trial is ongoing to address this question.

For pregnant women with a low risk of thrombophilia like asymptomatic heterozygous FVL or heterozygous prothrombin gene, postpartum clinical vigilance only is reasonable even with a family history of thrombosis because the postpartum VTE risk does not exceed 3%; but it is important for the clinician to review the risk threshold and have the patient decide if the 1% to 3% risk of postpartum VTE is of greater concern than the ~3% risk of major bleeding.

The authors' approach to thromboprophylaxis in the pregnant patient with a history of VTE or hereditary thrombophilia based on a synthesis of various guidelines is presented in Tables 3-9 and 3-10.

Thrombophilia and pregnancy complications

Historically, a number of pregnancy complications have been linked to thrombophilic states. Adverse pregnancy outcomes, however, are not uncommon in the general population, with up to a 15% rate of miscarriage and a 5% rate of 2 or more pregnancy losses. The association between thrombophilia and pregnancy loss has been confirmed in a number of case-control studies for women with thrombophilia, but it has not been confirmed in methodologically stronger cohort studies. Although a single late fetal loss and severe preeclampsia are associated with inherited thrombophilia, fetal growth restriction and placental abruption are not. In a meta-analysis of 25 studies, mostly case-control studies other than homozygous FVL and homozygous prothrombin 20210G, the pooled risk for pregnancy loss was equivocal. More rigorous studies that eliminate patients with previousVTE or VTE in pregnancy from the analysis do not support significant risk of recurrent pregnancy loss. Neither has there been any demonstrated association between thrombophilia and preeclampsia, placental abruption, or fetal growth restriction. As for the role of LMWH in the prevent-

Table 3-9 Outpatient antepartum prophylaxis

Clinical history	Anticoagulation
Multiple prior VTE	Treatment-dose LMWH or UFH
Prior VTE with high-risk thrombophilia	
Prior VTE with acquired thrombophilia	
Idiopathic prior VTE	Prophylactic-dose LMWH or UFH
Prior VTE with pregnancy or oral contraceptive	
Prior VTE with low-risk thrombophilia	
Family history of VTE with high-risk thrombophilia	
High-risk thrombophilia (including acquired)	
Low-risk thrombophilia	No treatment
Prior VTE provoked (eg, non-hormonal-trauma or postoperative)	
Low-risk thrombophilia and family history of VTE	

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Low risk: FVL or PGM heterozygous; PS def; PC def. High risk: FVL or PGM homozygous; FVL and PGM double heterozygous.

FVL, factor V Leiden; LMWH, low-molecular-weight heparin; PC, protein C; PGM, prothrombin gene mutation; PS, protein S; UFH, unfractionated heparin; VTE, venous thromboembolism.

tion of recurrent pregnancy loss in thrombophilic patients, a recent randomized trial (the TIPPS trial) showed lack of benefit with antepartum dalteparin in pregnant women with thrombophilia and previous placenta-mediated pregnancy complications. A subsequent meta-analysis of 963 eligible women with or without thrombophilia in 8 randomized trials of LMWH to prevent recurrent placenta-mediated pregnancy complications showed no benefit in women with a history of previous pregnancy that had been complicated by 1 or more of the following: preeclampsia, placental abruption, birth of a small for gestational age neonate [<10 th percentile], pregnancy loss after 16 weeks of gestation, or 2 losses after 12 weeks of gestation).

Recommendations. For women with a history of pregnancy complications, screening for inherited thrombophilia is not recommended and, for this group, neither is antithrombotic prophylaxis recommended. For women with inherited thrombophilia and a history of pregnancy complications, antithrombotic prophylaxis is not recommended, particularly given the recent results of the TIPPS trial, which showed lack of benefit with antepartum dalteparin in pregnant women with thrombophilia and previous placenta-mediated pregnancy complications.

Table 3-10 Postpartum VTE prophylaxis

Clinical history	Anticoagulation
Multiple prior VTE	6 wk of treatment-dose LMWH or UFH
Prior VTE with high-risk thrombophilia	
Prior VTE with acquired thrombophilia	
Idiopathic prior VTE	6 wk of prophylactic-dose LMWH or UFH
Prior VTE with pregnancy or oral contraceptive	
Prior VTE with low-risk thrombophilia	
Family history of VTE with high-risk thrombophilia	
High-risk thrombophilia (including acquired)	
Prior VTE provoked*	
Low-risk thrombophilia and family history of VTE*	
Low-risk thrombophilia	No treatment

Reproduced from D'Alton ME et al, *Obstet Gynecol*. 2016;128(4):688–698, with permission.

Low risk: FVL or PGM heterozygous; PS def; PC def. High risk: FVL or PGM homozygous; FVL and PGM double heterozygous.

*Changes from initial assessment.

FVL, factor V Leiden; LMWH, low-molecular-weight heparin; PC, protein C; PGM, prothrombin gene mutation; PS, protein S; UFH, unfractionated heparin; VTE, venous thromboembolism.

Antiphospholipid antibody syndrome (APAS)

The strongest evidence of an association between thrombophilia and fetal loss comes from studies in patients with antiphospholipid antibodies (APLAs). A diagnosis of antiphospholipid antibody syndrome (APAS) requires both laboratory and clinical criteria based on the Sapporo/Sydney meetings of the International Society of Haemostasis and Thrombosis. The clinical criteria require either:

1. Vascular thrombosis: 1 or more clinical episodes of arterial, venous, or small vessel thrombosis, or
2. Pregnancy morbidity:
 - (a) 1 or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus; or
 - (b) 1 or more premature births of a morphologically normal neonate before the 34th week of gestation because of:
 - (i) eclampsia or severe preeclampsia defined according to standard definitions; or

- (ii) recognized features of placental insufficiency; or
- (c) 3 or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

The laboratory criteria require the presence of lupus anticoagulant or moderate-to-high titer antibodies to immunoglobulin G (IgG) or immunoglobulin M (IgM); anticardiolipin (>40 GPL or MPL or greater than the 99th percentile); or IgG or IgM beta-2-glycoprotein I (greater than the 99th percentile) on 2 occasions at least 12 weeks apart. Correlation of APLAs with fetal growth restriction or placental abruption remains controversial. Among women with recurrent fetal loss (greater than or equal to 3 miscarriages), 15% have APLAs. Although the majority of fetal losses in normal individuals and patients with APLA occur early in the first trimester, an increased proportion of APLA-positive patients experience late fetal loss (after the 10th week of gestation). Inherited thrombophilia is less strongly associated with pregnancy loss than APLA. Several randomized studies, none of which was placebo controlled, have examined the effect of treatment of women with APLAs with aspirin, heparin, or both. These studies, which have been small and with heterogeneous criteria, generally have demonstrated an advantage of aspirin and heparin over either aspirin or heparin alone, although a randomized trial was stopped early when it became evident that LMWH and aspirin offered no advantage over aspirin alone, with almost 80% of women in both arms having successful pregnancies.

Recommendations. For women who fulfill laboratory and clinical criteria for APAS, antepartum prophylactic- or intermediate-dose UFH or prophylactic LMWH combined with LDA, 75 to 100 mg daily, is recommended but pending further data comparing LMWH+ASA versus ASA alone, ASA alone is an option. Antiphospholipid antibody screening is recommended in women with recurrent pregnancy loss (greater than or equal to 3 miscarriages before 10 weeks, or 1 beyond 9 weeks).

Assisted reproductive technology (ART)

Approximately 1 in 6 couples experience infertility. In achieving a subsequent successful pregnancy, assisted reproductive technology (ART) is employed, primarily in vitro fertilization. ART is coupled with ovarian follicle stimulation by gonadotropins and gonadotropin-stimulating hormones. Despite this, successful pregnancy results in only 35% to 40% of attempts (cycles). Thrombophilia has been

proposed as a potential mechanism in cases of failed reproduction. Case-control studies have indicated a 3-fold risk of failure in the presence of FVL mutation or APLA, but cohort studies have not substantiated either finding. Furthermore, antithrombotic therapy with aspirin or LMWH does not appear to appreciably increase the success rate.

On the other hand, ART is associated with an increased relative risk of VTE in the 3-fold range but with a low absolute risk. Arterial events also have been reported. The risk of thrombosis is increased if concurrent ovarian hyperstimulation syndrome (OHSS) develops. OHSS occurs in a third of cycles and is characterized by abdominal pain, bloating, and fluid retention. OHSS is present in 90% of the arterial events at a median of 11 days postembryo transfer, and in approximately 80% of the venous events, at a median of 42 days postembryo transfer. Most venous events occur in the neck or arm veins.

Management. Several guidelines advise prophylactic LMWH in severe OHSS, withholding LMWH for 12 to 24 hours before oocyte retrieval, then resuming 6 to 12 hours after retrieval and continuing for 3 months. Prophylaxis also is appropriate in nonsevere OHSS if there is high-risk thrombophilia (homozygous FVL or prothrombin gene mutation [PTGM]) or low-risk thrombophilia with a family history of thrombosis.

Anticoagulation issues during pregnancy

A number of scenarios exist concerning the prevention and treatment of primary and recurrent thrombosis in pregnant individuals. In general, when anticoagulation is indicated, the agent of choice in pregnancy—as previously mentioned—is LMWH, but a postpartum VKA is an option as opposed to continued LMWH. However, among women with protein C or S deficiency, postpartum VKAs should be used very cautiously with adequate bridging with LMWH, given the recognized low level of protein S due to pregnancy alone. In women with valvular heart disease, VKAs ideally should be avoided at least during weeks 6 through 12 of pregnancy. Insufficient data exist regarding safety or potential teratogenic effects of the new oral anti-Xa and new oral thrombin inhibitors to recommend their use in pregnancy.

Recommendations. In pregnant women, LMWH is the preferred antithrombotic agent, as discussed above. See also Table 3-11 for dosing details.

Oral vitamin K antagonists

Several toxicities of anticoagulant therapy unique to pregnancy must be considered when developing anticoagulation treatment approaches. First, the oral VKA warfarin is teratogenic, causing an embryopathy consisting of nasal hy-

Table 3-11 Unfractionated heparin and LMWH dosing

Prophylactic dosing	First trimester		Second trimester		Third trimester
UFH	5,000 U twice daily		7,500–10,000 U twice daily		10,000 U twice daily
Prophylactic LMWH dosing	<50 kg	50–90 kg	91–130 kg	131–170 kg	>170 kg
Enoxaparin	20 mg/d	40 mg/d	60 mg/d*	80 mg/d*	0.6 mg/kg/d*
Dalteparin	2,500 U/d	5,000 U/d	7,500 U/d	10,000 U/d	75 U/kg/d
Tinzaparin	3,500 U/d	4,500 U/d	7,000 U/d	9,000 U/d	75 U/kg/d
Therapeutic dosing	Initial dose		Adjusted target		
UFH	10,000 U twice daily		aPTT 1.5–2.5 × baseline 6 h after injection		
Therapeutic LMWH dosing	Initial dose		Adjusted target		
Enoxaparin	1 mg/kg twice daily		Twice-daily dosing: anti-factor Xa 0.6–1 U/ml 4–6 h after dose†		
Dalteparin	200 U/kg daily		Once-daily dosing: anti-factor Xa > 1 U/ml 4–6 h after dose†		
Tinzaparin	175 U/kg daily		Once-daily dosing: anti-factor Xa > 1 U/ml 4–6 h after dose†		

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*May be given in 2 divided doses.

†If anti-factor Xa level monitoring is indicated.

aPTT, activated partial thromboplastin time; LMWH, low-molecular-weight heparin; UFH, unfractionated heparin.

poplasia or stippled epiphyses and limb hypoplasia. The frequency of these abnormalities is estimated to be between 4% and 10%. The teratogenic effects occur primarily following exposure to warfarin during weeks 6 to 12 (primarily at 6 to 9 weeks) of gestation, whereas warfarin is probably safe preconception and during the first 6 weeks of gestation. VKAs used at any time during pregnancy have been associated with rare central nervous system developmental abnormalities, such as dorsal midline dysplasia and ventral midline dysplasia, leading to optic atrophy. Finally, an increased risk of minor neurodevelopmental abnormalities may occur in the offspring of women exposed to warfarin during the second and third trimesters, although the significance of these problems is uncertain. Warfarin may cause a dose-dependent anticoagulant effect in the fetus, which may lead to bleeding at delivery. In at least one series, warfarin was found to increase the rate of miscarriage, leading to the recommendation that heart valve patients receiving VKA during the second trimester of pregnancy should be switched to heparin beginning at 36 weeks of pregnancy.

Recommendation. For pregnant women, LMWH is the preferred antithrombotic agent.

Heparin-induced thrombocytopenia

HIT is an uncommon problem in pregnancy, but with only case series and anecdotal reports, the exact frequency is not known but estimated to be <0.1% with LMWH. It is higher, between 0.1% to 1%, if exposed first to UFH before LMWH. The mechanism by which HIT occurs is binding

of antibodies directed against the heparin-platelet-factor 4 (HPF4) complex, so-called HPF4 antibodies, which result in thrombocytopenia, with typically a greater than 50% drop below baseline platelet count. The risk of thrombosis is as high as 50% in patients with HIT; and thus, anticoagulation is critical to prevent thrombosis or pregnancy-related thrombotic complications. Although up to 50% of cardiac surgical patients develop HIT, prospective case series evaluating HPF4 antibody in pregnancy have reported low rates of HPF4 seroconversion and low rates of HIT. In pregnant patients with HIT, danaparoid, if available (eg, Canada, Japan, Europe, Australia), is considered to be the first-line therapy (Table 3-7). It does not appear to cross the placenta. In the United States, in lieu of danaparoid which is not available, fondaparinux is the best option but it does cross the placenta. However, the umbilical anti-Xa levels are subtherapeutic in the fetus at one-tenth the maternal level; but it must be stopped to allow safe anesthesia and delivery. The clinician should be reminded that safety data from first-trimester exposure are lacking.

Recommendations. Most guidelines suggest a platelet count 4 to 7 days after being on SUH or LMWH. For pregnant women with HIT, antithrombotic therapy with danaparoid is advised; when it is not available, as in the United States, fondaparinux in place of heparin or LMWH is recommended.

Heparin-associated osteoporosis

Prophylactic UFH is associated with a substantial risk of osteoporosis and a 2% incidence of vertebral fractures

when administered throughout pregnancy. Several reports suggest less osteoporosis occurs in patients who receive LMWH, but robust prospective comparison studies are lacking.

Recommendation. For pregnant women, LMWH is recommended as the preferred antithrombotic agent, as noted above.

Heparin-associated skin reactions

Skin reactions, varying from type 1 urticarial eruptions to type IV delayed hypersensitivity reactions, have been reported in 0.3% to 0.6% of patients receiving heparin. However, in at least one prospective study of 66 pregnant women, 29% reported pruritus, local erythema, and (less commonly) subcutaneous infiltrates and pain at the injection site. In pregnant women without signs of a type I reaction, switching to another LMWH preparation is recommended. In approximately one-third in whom a skin reaction recurs after switching from one LMWH preparation to another, switching to fondaparinux is recommended.

Recommendations. For pregnant women without signs of a type 1 reaction, switching to another LMWH preparation is recommended. In the approximately one-third in whom skin reactions recur after switching from one LMWH preparation to another, fondaparinux is recommended.

Mechanical heart valves

Without anticoagulant therapy, patients with mechanical heart valves have a high risk of arterial thromboembolism. Warfarin appears to be more effective than heparin in preventing valvular thrombosis in these patients but carries the highest rate of fetal complications. Debate continues, however, as to whether the benefit in prevention of valvular thrombosis in the mother offsets the risk of warfarin-induced embryopathy, increased miscarriage rate, and neurodevelopmental abnormalities in the fetus. In a recent systematic review of 800 pregnancies from 18 studies, the composite maternal risk (maternal death, prosthetic valve failure, and systemic thromboembolism) with VKA was 5% compared with 16% with LMWH, but the fetal risk (spontaneous abortion, fetal death, and the presence of any congenital defect) with VKA was 39% with VKA as compared to 13% with LMWH. It does appear, however, that most, if not all, of the thromboembolic events were due to subtherapeutic LWMH dosing, either because of inadequate dosing, lack of monitoring, or poor patient compliance.

Recommendations. For pregnant women with prosthetic heart valves, guidelines diverge between the 2012 ACCP

and the 2014 AHA/ACC. ACCP advises consideration of 3 approaches: (1) adjusted-dose LMWH throughout pregnancy; (2) adjusted-dose UFH throughout pregnancy; or (3) LMWH or UFH until the 13th week, with substitution of VKAs until close to delivery, at which time LMWH or UFH is resumed. For women judged to be at high risk for thromboembolism, such as older generation prosthesis in mitral position or previous thromboembolism, VKAs are recommended throughout pregnancy with replacement by LMWH or UFH close to delivery. In women with prosthetic valves at high risk of thrombosis, aspirin, 75 to 100 mg daily, is also recommended. The 2014 AHA/ACC guidelines advise as a Class 1 recommendation (ie, benefit >>> risk) the use of VKA in the second and third trimesters. For the first trimester, the guidelines stress that the complications of VKA for both mother and fetus are dose-dependent, with fewer adverse events when doses of less than or equal to 5 mg warfarin are used. Consequently, as a Class IIa recommendation (ie, benefit >> risk), continuation of warfarin is reasonable in the first trimester when doses ≤ 5 mg are used; otherwise, LMWH should be used in the first trimester. When LMWH is used, an adjusted dose based on anti-Xa levels to a target of 0.8 to 1.2 IU/mL 4 to 6 hours after a dose is recommended, with monitoring at least every 2 weeks. Weight-based dosing is not recommended. Although no guidelines exist for dose adjustment based on trough anti-Xa levels, some studies suggest a target anti-Xa level of 0.6 to 0.7 IU/mL.

Other thrombotic conditions in women

Ovarian vein thrombosis (OVT)

Ovarian vein thrombosis (OVT) is a relatively uncommon event, complicating approximately 1 per 600 to 1 per 2,000 pregnancies, most often in the postpartum period, and associated with CS delivery. Symptomatic OVT typically presents with fever and lower abdominal pain within the weeks following delivery. Complications of symptomatic OVT include sepsis, thrombus extension (25% to 30%) to the inferior vena cava or left renal vein, or (rarely) pulmonary embolism. Asymptomatic OVT is more common and may be benign, with a 30% incidence of pelvic vein thrombosis reported on screening MRI following vaginal delivery, and 80% detected in the nonpregnant population after major gynecologic surgery. Management guidelines are limited by a paucity of studies in the literature.

Recommendations. Anticoagulant therapy is indicated for patients with symptomatic postpartum OVT albeit based on limited evidence, and antibiotics should be used adjunctively when infection is suspected. Asymptomatic

OVT in the postpartum period and in the general population usually do not require treatment, which should generally be reserved for symptomatic OVT cases. Anticoagulant therapy should be given to patients with symptomatic OVT, and antibiotics should be instituted when infection is suspected. Optimal duration of anticoagulation has not been defined, and therapy duration has ranged from 11 days to 16 months. The most common duration of therapy has been 3 months. The utility of thrombophilia testing and follow-up imaging is unclear at this time. In general, testing has been reserved for cases of idiopathic VTE.

Pregnancy-related superficial thrombophlebitis

Superficial thrombophlebitis refers to the presence of a thrombus within a vein, diagnosed via duplex ultrasound, while superficial vein thrombosis typically refers to thrombosis of the axial veins (such as the great saphenous vein or the small saphenous vein). Typically, thrombophlebitis in the lower extremity refers to the presence of symptoms of venous inflammation and confirmed thrombosis of tributary veins. There is up to a 10% chance of DVT or PE once a superficial thrombosis develops.

Recommendations. Treatment of superficial thrombophlebitis is similar in pregnancy and nonpregnancy settings. Generally, treatment may vary according to whether thrombosis affects the axial veins or tributaries, and the presence or absence of other complications such as infection. In uncomplicated cases, particularly if the involved segment is ≤ 5 cm, initial management is supportive and consists of extremity elevation, warm or cool compresses, possibly compression therapy and serial duplex ultrasound to monitor for progression.

We recommend a low threshold for instituting anticoagulation in the settings where there is a significant risk of clot propagation into the deep system during pregnancy. Patients with increased risk for systemic thromboembolism include those with a thrombus in proximity (≤ 5 cm) to the deep system (especially if it involves the great saphenous vein), the effective vein segment is ≥ 5 cm, and those patients with other risk factors for thrombosis such as associated varicose veins, morbid obesity, or genetic thrombophilia.

The approach concerning dose and duration of LMWH for superficial thrombophlebitis in pregnancy resembles the decision-making process relating to prophylactic and therapeutic dosing for pregnancy-associated thromboembolism. Duration can be as short as 2 to 6 weeks if in the first trimester and longer if in the third trimester. Compression therapy is generally indicated with pregnancy-specific compression stockings.

Hematologic health issues in the premenopausal woman

Bleeding in the premenopausal woman

Bleeding disorders in women are underrecognized and undertreated conditions. Hemophilia, the most widely known and studied bleeding disorder, is a disease of males. Women, however, are as likely as men to have bleeding disorders other than hemophilia and are in fact disproportionately affected by these diseases due to the bleeding challenges of menstruation and childbirth. Because bleeding disorders in women tend to be less severe and specific than hemophilia, it is more difficult for physicians and patients to recognize symptoms and diagnose these conditions. In one national survey of 75 women with VWD, the average time from onset of bleeding symptoms to diagnosis was 16 years. This section reviews the most common gynecologic manifestations of bleeding, as well as recommendations for the laboratory evaluation and management of women presenting with excessive bleeding.

Heavy menstrual bleeding

Heavy menstrual bleeding (HMB; the current terminology, replacing the term menorrhagia) can be defined as passing large clots (the size of a quarter or larger) or prolonged bleeding (longer than 7 days and/or requiring change of a tampon or pad more frequently than every 2 hours) resulting in the loss of >80 ml of blood per menstrual cycle. HMB is the most common gynecological complaint, affecting 10 million American women each year (ie, approximately 1 in 5 women). In 2007, the International Federation of Gynecology and Obstetrics developed a useful construct in classifying HMB in terms of the acronym PALM-COEIN (polyps, adenomyosis, leiomyoma, malignancy and hyperplasia; coagulopathy, ovulatory dysfunction, endometrial, iatrogenic, and not yet classified).

The underlying cause of HMB is frequently undiagnosed. HMB can negatively affect quality of life, sometimes leading to hysterectomy in reproductive-aged women who may have otherwise been able to be medically managed, potentially preserving their fertility, if desired. Not surprisingly, HMB can lead to iron deficiency and chronic anemia. Women with HMB have significantly lower perceived general health and poorer quality of life in terms of their ability to fully participate in school, work, sports, and social activities.

It is well established that HMB is the most common bleeding symptom among women with bleeding disorders, occurring in up to 80% to 90% of patients, and that bleeding disorders are common among women present-

ing with HMB. Therefore, it is imperative that physicians screen for underlying bleeding disorders when evaluating an adolescent or woman with HMB. Up to 11% to 16% of women with HMB and a normal gynecologic exam have VWF deficiency. A recent opinion issued by the Adolescent Health Committee supports screening for VWD in adolescents presenting with severe HMB. However, it is important to consider that VWF levels may be affected proportionately by aging, stress, inflammation, anemia, pregnancy, and high-dose oral contraceptives; while levels can be lower than expected if sampled in the follicular phase of the menstrual cycle and falsely low due to sample processing. Even in the presence of gynecologic disease, such as anovulatory bleeding in adolescence or fibroids in perimenopause, an underlying bleeding disorder may be an additional contributing factor to HMB and should be considered in the evaluation. In summary, an inherited bleeding disorder should be considered if any of the following indicators are present: (1) HMB since menarche, (2) family history of a bleeding disorder, or (3) a personal history of 1 or more additional bleeding symptoms.

Because HMB is such a frequent problem, it would be cost-prohibitive to screen all women with it for underlying bleeding disorders. Identifying patients with either "severe or significant" HMB is challenging, given that actual measurement of menstrual blood loss is not feasible in clinical practice. Therefore, an active area of research has been the development and validation of bleeding assessment tools in this field. One of the first tools developed was the Pictorial Blood Assessment Chart (PBAC), first published in 1990. To complete the chart, women compare both the number and degree of saturation of pads and tampons with those depicted on a chart (Figure 3-5).

A total score of >100 per menstrual cycle is associated with menstrual blood loss of >80 mL (definition of

HMB). A major limitation of this tool is that it must be completed prospectively, so results are not available at the time of initial evaluation. Moreover, completion of the score after the evaluation may be limited by subjective bias as well as poor compliance. In a study of 226 women who consented to formal measurement of menstrual blood loss, variables that predicted blood loss of >80 mL were changing a pad or tampon more than hourly, passing clots >1 inch in diameter, and low ferritin.

More recently, a screening tool developed by Philipp et al may identify women with HMB who are more likely to have an underlying bleeding disorder. The tool contains 8 questions in 4 categories: (1) severity of HMB, (2) family history of bleeding disorder, (3) personal history of excessive bleeding, and (4) history of treatment for anemia (Table 3-12). The screen is considered positive if an affirmative response is obtained in any 1 of the 4 categories. The sensitivity of this tool for underlying hemostatic defects in adult women is 89%, which increases to 93% to 95% with a serum ferritin level of ≤ 20 ng/mL and a PBAC score of >185, respectively. A variety of more general bleeding assessment tools, most modified based on the original Vicenza Bleeding Questionnaire, and including a consensus bleeding assessment tool set forth by the International Society of Thrombosis and Haemostasis, have been developed. However, the sensitivity and specificity of these instruments in identifying underlying bleeding disorders in women with HMB have not been formally studied. It is important to recognize that because of increased proliferation of the endometrium, menstrual bleeding may be even heavier during anovulatory cycles. For this reason, HMB in women with inherited bleeding disorders often presents at menarche and may be particularly troublesome during the premenopausal years. As a result, more aggressive or combination therapy may be required during these time periods.

Figure 3-5 Pictorial chart assessment of menstrual flow. A total score of >100 points (pts) is consistent with menorrhagia (80% sensitivity and specificity); >185 has >85% PPV and PNV. Adapted from Janssen CA et al, *Obstet Gynecol*. 1995;85(6):977–982.

The numbers 1-8 represent the consecutive days of your menstrual period. Please record, for each day, the number of pads you used that match each illustration									
	Pad	1	2	3	4	5	6	7	8
1 pt/pad									
5 pts/pad									
20 pts/pad									
	Clots (Yes/No)								

The numbers 1-8 represent the consecutive days of your menstrual period. Please record, for each day, the number of tampons you used that match each illustration									
	Tampon	1	2	3	4	5	6	7	8
1 pt/tampon									
5 pts/tampon									
10 pts/tampon									
	Clots (Yes/No)								

Table 3-12 Screening tool for inherited bleeding disorders in women presenting with heavy menstrual bleeding

Screening questions	Score
Q1. How many days did your period usually last, from the time bleeding began until it completely stopped?	1 = ≥7 days 0 = <7 days
Q2. How often did you experience a sensation of “flooding” or “gushing” during your period?	1 = Every or most periods 0 = Never, rarely, or some periods
Q3. During your period did you ever have bleeding where you would bleed through a tampon or napkin in ≤2 hours?	1 = Every or most periods 0 = Never, rarely, or some periods
Q4. Have you ever been treated for anemia?	1 = Yes 0 = No
Q5. Has anyone in your family ever been diagnosed with a bleeding disorder?	1 = Yes 0 = No
Q6. Have you ever had a tooth extracted or had dental surgery?	1 = Yes, if had and bled 0 = No
Q7. Have you ever had surgery other than dental surgery? Q7a. Did you have bleeding problem after surgery?	See 7a. below 1 = Yes 0 = No
Q8. Have you ever been pregnant? Q8a. Have you ever had a bleeding problem after delivery or after a miscarriage?	See 8a. below 1 = Yes 0 = No

The screen is considered positive if an affirmative response is obtained in any 1 of the 4 categories covered by the 8 questions, including (1) bleeding severity, (2) family history of bleeding disorder, (3) personal history of excessive bleeding, and (4) history of treatment for anemia. Women with a positive screen should undergo comprehensive hemostatic testing to determine whether they have a bleeding disorder.

Scores are adapted from Philipp CS et al. *Am J Obstet Gynecol*. 2011;204:e1-209.e7; and Philipp CS et al. *Am J Obstet Gynecol*. 2008;198:e1-163.e38.

There are few published robust trials regarding the management of HMB, particularly in women with an underlying bleeding disorder. One practical approach is to first offer combined oral contraceptive (COC) or the levonorgestrel intrauterine device if contraception is concurrently desired. Additional options in women with an underlying bleeding disorder include intranasal DDAVP (Stimate) and oral TXA (Lysteda). Both TXA and DDAVP have been studied and both have demonstrated reduced menstrual flow and improved quality of life among females with HMB and abnormal laboratory hemostasis, but TXA proved to be more effective than IN-DDAVP. To further understand the efficacy and safety of these hemostatic agents for HMB, the study of combined therapy of IN-DDAVP and antifibrinolytic therapy or hormonal therapy is needed. (Figure 3-6). The management of HMB in adolescents presents some additional challenges, as the etiol-

ogy is often multifactorial, and patients or parents may be reluctant or unwilling to use the hormonal preparations recommended as first-line therapy.

COC containing both estrogen and progestin are available in oral, transdermal, and vaginal ring formulations. These agents reduce menstrual loss by inducing changes that thin the endometrium. Several studies have demonstrated that COCs increase fibrinogen, prothrombin, and factor VII; and consequently promote hemostasis. It is unknown whether the increase in coagulation factors contributes to the clinical response, but these agents do reduce menstrual blood loss and increase hemoglobin in women with anemia. Additional benefits of hormonal management of HMB include cycle regulation, decreased dysmenorrhea, and improvement in acne. In a trial of combined contraceptive hormones in 14 adolescents with VWD, menstrual blood loss measured by PBAC decreased

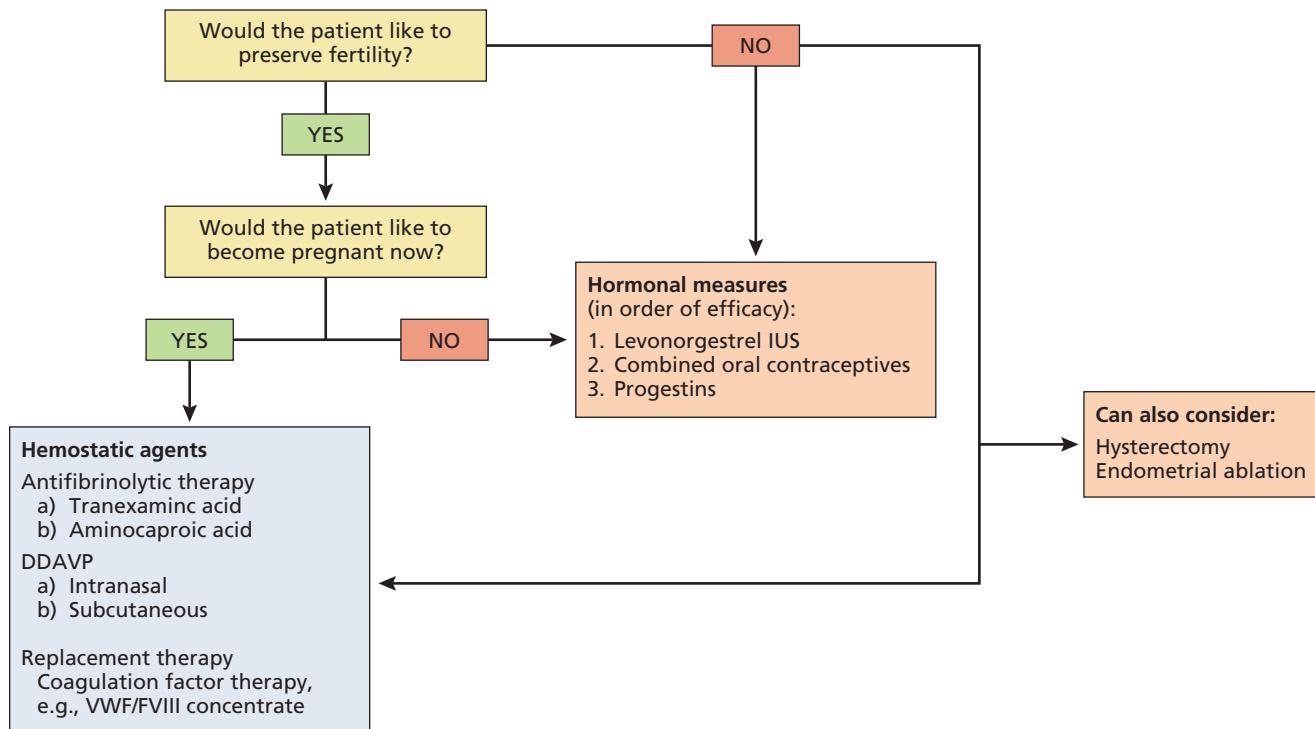


Figure 3-6 Suggested algorithm for management of bleeding disorder-related HMB. Adapted from James AH et al, *Am J Obstet Gynecol*. 2009;201:12 e1–e8.

in 12 of 14 patients. Although these agents are generally well tolerated in adolescents, there may be hesitancy to use them, particularly in the families of young, sexually abstinent adolescents, and time should be allotted for thorough discussion and education. Extended cycling or continuous regimens of COCs can be particularly helpful in reducing menstrual blood loss, especially in the setting of anemia. Breakthrough bleeding is a possible adverse effect of these regimens, especially in adolescents.

The LNG-IUS (levonorgestrel-intrauterine system) is a progestin-impregnated intrauterine device that reduces menstrual blood loss by opposing the estrogen-induced growth of the endometrium. The short-term and long-term efficacy of this device has been demonstrated in a small cohort of adult women with inherited bleeding disorders in the United Kingdom. A recent study has shown improvement in HMB in 13 adolescents with HMB and an underlying bleeding disorder who failed prior hormonal therapy, and the majority previously failed hemostatic therapy. Follow-up of such patients is important, as a Canadian retrospective study of 20 women with HMB and an underlying bleeding disorder post-LNG-IUS placement reported that half necessitated removal of the device because of patient dissatisfaction, malposition, or expulsion. Larger prospective studies of complication

rates and effectiveness of premedications (eg, DDAVP and/or TXA) or prolonged use of antifibrinolitics in preventing these complications are needed. Physician-patient discussions regarding this device also require substantial time for education, as patients often have misperceptions that these devices cannot be removed easily once placed, can be placed only in women who have had children, or perhaps are even limited to those who have completed their planned childbearing. Patients should also be informed of a risk of prolonged spotting and increased cyst formation from unopposed progesterone exposure. Other progestin-only contraceptives, such as depot medroxyprogesterone acetate (Depo-Provera), progestin-only pills, and the etonogestrel implant (Implanon, Nexplanon), also reduce endometrial proliferation and therefore menstrual blood loss. Insertion of the implant might cause bleeding in a woman with a bleeding disorder, and the use of a preprocedure hemostatic agent should be considered.

Recommendations. Females with HMB since menarche or a family or personal history of bleeding should be screened for a bleeding disorder. For women with HMB, COCs containing both estrogen and progestin are recommended first-line therapy (Table 3-9). Alternative approaches include the LNG-IUS or other progestin-only

contraceptives, including Depo-Provera, progestin-only pills, or subcutaneous implants. Nonhormonal therapies include intranasal desmopressin and antifibrinolytics. For patients with type 3 VWD or other severe factor deficiencies, VWF or other clotting factor concentrates during menses may be considered.

Hemorrhagic ovarian cysts and endometriosis

The second most common reproductive tract bleeding manifestation is hemorrhagic ovarian cysts, which occur more commonly in women with VWD, platelet function defects, and rare bleeding disorders than in women without bleeding disorders. Ovarian cysts develop when bleeding occurs in the residual follicle after an ovum is extruded. In the acute setting, surgery, TXA, and clotting factor replacement have been used to manage hemorrhagic ovarian cysts. COCs, which reduce the likelihood of ovulation and increase clotting factors, are used to prevent recurrences. Even among women with bleeding disorders but without documented hemorrhagic ovarian cysts, there is a high prevalence of midcycle pain or “mittelschmerz,” a phenomenon that is thought to be associated with bleeding at the time of ovulation.

Women with bleeding disorders also are diagnosed more frequently with endometriosis. In one case-control study, endometriosis was reported in 30% of women with VWD as compared with 13% in the control group. The etiology of this phenomenon is unclear, but one hypothesis is that women with HMB are at higher risk of retrograde menstrual bleeding (reflux of menstrual blood out of the uterine cavity), which then stimulates the development of endometrial tissue implants in the fallopian tubes or peritoneal cavity. Alternatively, women with bleeding disorders may not be more likely to develop endometriosis but simply are more likely to present with symptomatic bleeding, or they are more likely to experience hemorrhagic cysts that are misdiagnosed as endometriosis. Similarly, the development of fibroids, polyps, and endometrial hyperplasia may unmask a previously subclinical bleeding tendency and cause problematic bleeding, so that the diagnosis of these conditions (but likely not the true frequency) becomes more common in women with bleeding disorders. As a result of all of these manifestations, women with bleeding disorders are more likely to undergo hysterectomy than their peers and more likely to undergo the procedure at an earlier age.

Recommendations. For females who develop hemorrhagic ovarian cysts in the setting of an underlying bleeding disorder, clotting factor replacement alone, or together with TXA, is recommended for acute management, and COCs are recommended to prevent recurrence.

Thrombosis and oral contraceptives in the premenopausal woman

Hormonal agents are commonly used by >100 million premenopausal women in the United States in the form of contraceptive agents, which are available in oral, transdermal, and vaginal ring formulations. The most common formulation is the oral combination of estrogen and progestin, “combined” OC (COC). Progestin-only agents are as effective as estrogen-progestin combination agents and are available in oral, intramuscular, intrauterine, and subdermal forms. Over 40 case-control studies, prospective cohort studies, and randomized trials of women using OC provide estimates of the risk of VTE to be 2- to 3-fold greater than in nonusers; although the absolute risk is low, 2 to 4 per 10,000 person-years of OC use. This 2- to 3-fold increased risk is much lower than the 5- to 10-fold increased risk seen during pregnancy and the 15- to 35-fold increased risk during the postpartum period. The risk of VTE is highest in the first year of use, especially in the first 3 months. The risk dissipates 1 to 3 months after discontinuation.

Several studies indicate that the VTE risk in heterozygous carriers of FVL or prothrombin G20210A is greater than would be expected if the risks were additive; that is, 28 to 50 per 10,000 woman-years of COC use. In women with protein C or antithrombin III deficiency, the absolute VTE risk with COC use is reported to be even higher: 400 per 10,000 patient-years of COC use.

The increased risk of thrombosis has been attributed to the estrogen component of contraception preparations. Estrogen increases procoagulants, such as factor VIII, VWF, and fibrinogen, and decreases fibrinolytic activity and natural anticoagulants, such as protein S. Evidence has now accumulated that the negative influence of COCs on the anticoagulant protein C pathway leads to acquired protein C resistance, and this is thought to be a primary mechanism underlying the prothrombotic effect of these agents. Third-generation COCs (desogestrel) are associated with higher VTE risk, as are drospirenone-containing preparations, presumably because the progestin component has less of an anticoagulant effect than levonorgestrel of the second-generation preparations, although the estradiol dose is less (Table 3-13). Progestin-only contraceptives appear to confer lower VTE risk, but there have been no robust head-to-head clinical trials to confirm this. The vaginal ring and the transvaginal patch both contain estrogen and are associated with an increased risk of thrombosis relative to nonusers.

In addition to the small absolute risk of thrombosis with COCs, women may have underlying thromboembolic risk

Table 3-13 Risk of VTE associated with hormonal contraceptives

Contraceptive	Odds ratio	95% CI
COC 30 µg, desogestrel	7.3	3.30–10.00
COC 30 µg, levonorgestrel	3.6	1.75–4.60
Depo-Provera	3.6	0.70–1.50
Transdermal patch	2.2	0.70–3.80
Vaginal ring	1.6	1.02–2.37
Progestin-only pills	0.6	0.33–3.41
Levonorgestrel IUD	0.3	0.10–1.26

Adapted from Manzoli L et al. *Drug Safety*. 2012;35:191–205; Ueng J, Douketis JD. *Hematol Oncol Clin North Am*. 2010;24:683–694; van Hylckama Vlieg A et al. *BMJ*. 2009;339:b2921; Lidegaard O et al. *BMJ*. 2012;344:e2990; van Hylckama Vlieg A et al. *Arterioscler Thromb Vasc Biol*. 2010;30:2297–2300; van Vlijmen EF et al. *Blood*. 2011;118:2055–2061; WHO. Medical eligibility criteria for contraceptive use. 2008 Update. (http://whqlibdoc.who.int/publications/2010/9789241563888_eng.pdf); Mantha S et al. *BMJ*. 2012;345:34944.

COC, combined oral contraceptive; IUD, intrauterine device.

factors that augment the contraception-related thrombosis risk. For example, women who have hypertension, smoke, or are >35 years of age have higher risks of myocardial infarction (MI) and stroke. Diabetes and hypercholesterolemia also increase the risk of MI, while migraines with aura raise the risk of stroke. A history of prior VTE or complicated valvular heart disease may increase the risk of contraception-related thrombosis. Recently, an increased risk of VTE has been associated with polycystic ovarian syndrome.

Among users of hormonal contraception, obese women, smokers, and those with inherited thrombophilia are the patients at highest risk of VTE. WHO has categorized a large number of medical conditions according to the level of risk associated with a variety of contraceptive agents. The 4 categories established by WHO range from no restrictions (category 1) to unacceptable health risks (category 4). In 2003, the WHO added a new medical condition to the list of risk states for which COCs are nonpreferred contraceptive choices—any known inherited thrombophilia. Women who are considered to have an unacceptable level of thromboembolic risk with COCs may still be candidates for progestin-only contraceptives. With a few exceptions, the WHO classifies all of the risk states described above as category 3 or 4 with regard to COCs, but as category 1 or 2 with regard to progestin-only products (POCs). This remains unconfirmed with the lack of any trial assessing safety or efficacy of POCs.

Studies of coagulation factors during POC use have not identified clinically meaningful changes. In case-control studies investigating the association between oral POC and VTE, the risk of VTE was not significantly greater in POC users than in nonusers (Table 3-13), and in a recent meta-

analysis there appeared to be no increased risk of MI with POC use. The latter study included small numbers of non-healthy POC users. Concern regarding a possible risk of VTE with POC may stem from reports that higher-dose progestins, used for other than contraception purposes, have been associated with VTE. Furthermore, an international study reported a possible increase in stroke risk in women with hypertension using injectable POCs. A study from the Netherlands reported a 3.6-fold increased risk of VTE in women using Depo-Provera, as compared with nonusers, but no increased risk in women using LNG-IUS. A recent meta-analysis concluded that the use of POCs was not associated with an increased risk of VTE compared to non-users. Thus, with a paucity of clinical trials, many controversies remain regarding optimal contraception in women at risk for thrombosis.

Recommendations. For premenopausal females with thrombophilia seeking contraception, the potential VTE risks associated with COCs should be weighed against the risk of unplanned pregnancy. In the absence of definitive trials, the risk benefit should be thoroughly discussed with the patient; in the case of asymptomatic FVL the VTE risk is 1:300. However, given efforts nationally in educating providers and patients on the use and efficacy of long-acting reversible contraception (LARC) such as the etonogestrel implant or copper intrauterine device or the levonorgestrel IUS with better tolerance and adherence than COCs, there is very little reason if any to offer COC to a high-risk VTE patient. However, there are several settings where COCs are beneficial and where the benefits of LARC have not been clearly demonstrated to date: hyperandrogenism (typically due to polycystic ovary syndrome), acne, ovarian cyst prevention, premenstrual syndrome, premenstrual dysphoric disorder, and pelvic pain. As such, when estrogen-containing contraception is preferable, theoretically, a potential option is co-administered antithrombotic therapy (eg, warfarin, LMWH, or direct oral anticoagulant) to reduce VTE risk. Interestingly, in the rivaroxaban licensure trial for DVT/PE, patients with COC-related VTE were given the option to discontinue OC (n=1475) or not (n=306). The risk of VTE was equivalent (4.0% in the continued COC cohort vs 3.8% in cohort having discontinued COC). However, there are no prospective safety data. In general, we recommend LARC for the patient with asymptomatic thrombophilia seeking hormonal contraception.

Conclusion

This chapter summarizes the most recent evidence-based guidelines available through the Council on Patient Safety in Women's Health Care, ACOG, ACCP, National Partner-

Table 3-14 Safety of medications during pregnancy and breastfeeding

Medication	Pregnancy category	Breastfeeding
Antiplatelet agent (clopidogrel [Plavix])	B	Safety unknown, caution advised
Antiplatelet agents (aspirin)	D	Possibly unsafe
Antithrombin III concentrate (Thrombate)	B	Safety unknown, caution advised
Azathioprine	D	Possibly unsafe
Cyclosporine	C	Safety unknown, caution advised
Deferoxamine (Deferal); deferasirox (Exjade, Jadenu)	C	Safety unknown
Direct oral anticoagulant (FXa inhibitors: apixaban, bretixaban, edoxaban, rivaroxaban)	C	Safety unknown
Eculizumab (Soliris)	C	Caution in nursing
Eltrombopag (Promacta)	C	Safety unknown, caution advised
Granulocyte colony-stimulating factor (G-CSF)	C	Caution in nursing
Hydroxyurea	D	Unsafe
Intravenous immunoglobulin (IVIg)	C	No/minimal risk
Low-molecular-weight heparin (enoxaparin)	B	Caution in nursing
Low-molecular-weight heparin (dalteparin)	B	Caution in nursing
Low-molecular-weight heparin (fondaparinux)	B	Caution in nursing
Corticosteroids (prednisone)	D	Probably safe
Factor (VII,VIII, IX, VWF)	C	Caution in nursing
Folic acid (dietary)	A	No/minimal risk
Oral iron: gluconate (Ferrlecit)	B	Caution in nursing
Parenteral iron: sucrose (Venofer)	B	Caution in nursing
Parenteral iron: dextran (fumoxitylol, Dexferrum, INFeD)	C	Caution in nursing
Recombinant erythropoietin (Epogen)	C	Caution in nursing
Recombinant factor (VII, VIII, IX)	C	Safety unknown, caution advised
Rho (D) immune globulin (RhoGAM)	C	Probably safe, caution advised
Rituximab (Rituxan)	C	Unsafe
Romiplostim (Nplate)	C	Safety unknown, caution advised
Vitamin K antagonists (warfarin [Coumadin])	D (mechanical valves); X (all other)	Caution in nursing
Vitamin K (phytadione)	C	Safe

For each drug, see package insert for drug-specific recommendations.

Pregnancy category A: safe for use in pregnancy. Pregnancy category B: animal studies show no risk or adverse fetal effects but controlled human first trimester studies are not available or do not confirm; no evidence of second, third trimester risk; fetal harm unlikely. Pregnancy category C: animal studies show adverse fetal effect(s) but there are no controlled human studies *or* no animal or human studies exist; weigh possible fetal risk vs maternal benefit. Pregnancy category D: positive evidence of human fetal risk; maternal benefit may outweigh fetal risk in serious or life-threatening situations. Pregnancy category X: contraindicated; positive evidence of serious fetal abnormalities in animals, humans, or both; fetal risks outweigh maternal benefit.

ship for Maternal Safety, NHLBI, NHF, and WHO to provide optimal care for women with blood disorders in pregnancy and premenopause. Although this compilation is up to date, new therapies and clinical trial findings will evolve to improve care and offer new and better approaches. A commitment by the hematologist to continually update and stay abreast of new evidence is critical to ensure optimal

work and interaction by the multidisciplinary team, which will translate into the highest quality of care and best outcomes for women with blood disorders.

For reference, Table 3-14 provides some guidance regarding safety of medications during pregnancy and breastfeeding, in the order the medications appear in the text.

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4

Hematopoietic growth factors

ALAN E. LICHTIN AND VINAY PRASAD

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Introduction

The hematopoietic growth factors (HGFs) and their receptors play essential roles in regulating hematopoiesis. Specific factors for each hematopoietic lineage are critical for producing and maintaining normal circulating levels of the cells. Granulocyte colony-stimulating factor (G-CSF) regulates neutrophil production; granulocyte-macrophage colony-stimulating factor (GM-CSF) enhances production of neutrophils, monocytes, and eosinophils; erythropoietin (EPO) regulates red blood cell production; and thrombopoietin (TPO) controls platelet production (see video in online edition). This chapter focuses on the results of clinical trials and approved uses for these HGFs and provides a glimpse of other factors involved in the early stages in development.

 The online version of this chapter contains an educational multimedia component on normal hematopoiesis.

Conflict-of-interest disclosure:

Dr. Prasad reports receiving royalties from his book *Ending Medical Reversal*, that his work is funded by the Laura and John Arnold Foundation, that he has received honoraria for Grand Rounds/lectures from several universities, medical centers, and professional societies, and is a writer for Medscape. Dr. Lichtin declares no competing financial interest.

Off-label drug use: Dr. Lichtin: epoetin alfa and darbepoetin alfa in myelodysplastic syndromes.

Myeloid growth factors

Granulocyte colony-stimulating factor (filgrastim, tbo-filgrastim, and lenograstim)

G-CSF is a myeloid growth factor produced by monocytes, macrophages, fibroblasts, endothelial cells, and a number of other types of cells. G-CSF plays the central role of regulating neutrophil formation and deployment. In healthy individuals, circulating levels of G-CSF are low or undetectable. A dramatic increase in the circulating levels of G-CSF occurs in the setting of infection and inflammation and with the administration of endotoxin or mediators of inflammation, such as interleukin-1 and tumor necrosis factor.

The biological effects of G-CSF are mediated through the G-CSF receptor expressed on both mature neutrophils and neutrophil progenitors (see video in online edition). G-CSF knockout mice with a targeted disruption of the G-CSF receptor develop severe neutropenia, whereas hematocrit levels and platelet counts are normal. Children with severe congenital neutropenia progressing to myelodysplasia or acute myeloid leukemia (AML) often have acquired mutations in the G-CSF receptor, most of which consist of truncation of the cytoplasmic tail of the receptor (see Chapter 16).

Available recombinant forms of G-CSF include filgrastim produced in *Escherichia coli* by the introduction of the human G-CSF gene. This form is identical to native human G-CSF except for the addition of an amino-terminal me-

thionine. Filgrastim is licensed for use in the United States and in many other countries (Table 4-1). An alternative nonglycosylated recombinant methionyl form of G-CSF, tbo-filgrastim, garnered US Food and Drug Administration (FDA) approval (Table 4-2). Lenograstim is a glycosylated form of G-CSF produced in a mammalian cell line and is not approved for clinical use in the United States.

Pegylated methionyl G-CSF (pegfilgrastim)

Pegfilgrastim is methionyl G-CSF with polyethylene glycol covalently bound to the amino terminal methionine residue. Importantly, pegylation reduces the renal clearance of G-CSF through stearic hindrance and prolongs its circulation and the duration of its effects. Clinical trials comparing pegylated G-CSF and G-CSF demonstrated similar biological activities and clinical benefits, including the duration of chemotherapy-induced severe neutropenia and occurrence of febrile neutropenia (FN). The pharmacokinetics of pegfilgrastim should not be affected by hepatic insufficiency, but it has not been evaluated adequately in this setting. Although less studied in children, the efficacy and safety of pegfilgrastim appears similar to that in adults. The FDA-approved indications for pegfilgrastim are shown in Table 4-3.

On 6 March 2015, the FDA approved the first biosimilar compound: filgrastim-sndz (Zarxio; Sandoz). Biosimilars are

to biological therapies what generics are to small molecular drugs—an interchangeable version intended to reduce the price of the medication. Filgrastim-sndz was approved on the basis of pharmacologic data showing equivalent pharmacokinetics and pharmacodynamics to filgrastim, as well as a clinical trial in human subjects which showed no clinically meaningful difference in the rate of FN between filgrastim and filgrastim-sndz.

Sandoz requested that the FDA extrapolate pathophysiological understanding, pharmacologic parameters, and demonstration of equivalence for one approved indication, to provide a drug label for filgrastim-sndz for all 5 approved filgrastim indications. The FDA granted the request, and Filgrastim-sndz became the first biosimilar to enter the US market, with the promise of large cost savings.

As of September 2017, those savings reportedly have been modest. Filgrastim-sndz is priced 15% below the price of the parent compound, according to Truven Health Analytics' Red Book. Others have argued that prices should continue to fall as more biosimilars enter the market, as has been the experience with generic drugs. Recently, Dave et al demonstrated that price reductions occur linearly with the number of generic manufacturers in the market. Biosimilars are poised to enter the US market for many of the compounds discussed in this chapter in the years to come.

Granulocyte-macrophage colony-stimulating factor (sargramostim, molgramostim)

GM-CSF is a glycoprotein constitutively produced by monocytes, macrophages, endothelial cells, and fibroblasts. GM-CSF production is enhanced by inflammatory cytokines such as interleukin-1 or tumor necrosis factor. GM-CSF promotes the growth of myeloid colony-forming cells, increases the number of circulating neutrophils and monocytes, and enhances the phagocytic function and microbicidal capacity of mature myeloid cells. GM-CSF also stimulates dendritic cell maturation, proliferation, and function, and it increases antigen presentation by macrophages and dendritic cells. That GM-CSF is not essential for hematopoiesis is confirmed by the demonstration of normal complete blood counts and normal number of marrow progenitor cells in GM-CSF knockout mice. Evidence exists, however, that GM-CSF plays a key role in the function of pulmonary macrophages. Mice that lack GM-CSF have lung pathology consistent with pulmonary alveolar proteinosis. Similarly, some cases of human pulmonary alveolar proteinosis are related to a defect in the common β -chain of the receptor for GM-CSF, IL-3, and IL-5. Infants that are so affected have decreased alveolar macrophage function and accumulate surfactant in the alveoli.

Table 4-1 FDA-approved indications for filgrastim

Accelerate neutrophil recovery in patients receiving myelosuppressive chemotherapy
Accelerate neutrophil recovery after acute myeloid leukemia induction or consolidation chemotherapy
Accelerate neutrophil recovery in patients following a bone marrow transplant
Mobilize peripheral blood stem cells
Severe chronic neutropenia (idiopathic, cyclic, congenital)

Table 4-2 FDA-approved indication for tbo-filgrastim

Reduction in the duration of severe neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs associated with a clinically significant incidence of febrile neutropenia
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Table 4-3 FDA-approved indication for pegfilgrastim

Decrease the incidence of infection as manifested by febrile neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs associated with a clinically significant incidence of febrile neutropenia

Table 4-4 FDA-approved indications for GM-CSF sargramostim

Reduce the risk of death due to infection in patients ≥ 55 years old undergoing induction chemotherapy for acute myeloid leukemia
Mobilize autologous peripheral blood stem cells and enhance neutrophil recovery after transplantation
Promote neutrophil recovery after autologous or allogeneic bone marrow transplantation
Improve neutrophil production in patients with delayed engraftment or graft failure after autologous or allogeneic bone marrow transplantation

Recombinant forms of GM-CSF available for clinical use include sargramostim derived from yeast and molgramostim expressed by *E. coli*. The sequence of sargramostim differs from that of native GM-CSF by a single amino-acid substitution at position 23. Only sargramostim is approved for clinical use by the FDA (Table 4-4).

Clinical use of G-CSF and GM-CSF

Prevention of chemotherapy-induced febrile neutropenia

The main clinical use of G-CSF and GM-CSF is for the prevention of FN (temperature $>38.3^{\circ}\text{C}$ with neutrophils less than $0.5 \times 10^9/\text{L}$) in patients receiving cancer chemotherapy. FN represents the major dose-limiting toxicity of cancer chemotherapy and is associated with considerable morbidity, mortality, and costs. The clinical use of G-CSF is based on results of numerous randomized controlled trials and meta-analyses of such trials and supported by clinical practice guidelines. FDA approval of G-CSF for prevention of FN was based on 2 pivotal randomized controlled trials in patients with small-cell lung cancer receiving intensive combination chemotherapy associated with prolonged severe neutropenia with a high risk of FN. Primary prophylaxis with G-CSF initiated within the first 3 days after chemotherapy and continued for up to 10 days reduced the duration of severe neutropenia to about 3 days and reduced the occurrence of FN and documented infection by 50%. A pivotal randomized trial in patients with breast cancer found tbo-filgrastim to be superior to placebo, and equivalent to filgrastim, in duration of severe neutropenia after chemotherapy.

The results of these trials have been confirmed in multiple other randomized controlled trials across a spectrum of malignancies and chemotherapy regimens, consistently demonstrating a reduction in the risk of FN in the initial cycle, as well as across repeated cycles of treatment. At the same time, little or no benefit from G-CSF administration has been observed when treatment is delayed until neutropenia is already present. Although individual studies were

not sufficiently powered to assess any impact on infection-related or all-cause mortality, meta-analyses of these trials have demonstrated a significant reduction in these complications with primary G-CSF prophylaxis in patients receiving conventional chemotherapy. These analyses also have demonstrated that G-CSF prophylaxis enables a greater percentage of patients to receive full-dose chemotherapy on schedule through the avoidance of neutropenic complications that lead to preemptive dose reductions or treatment delays. Meta-analyses of randomized controlled trials also suggest that G-CSF support of patients receiving cancer chemotherapy may improve long-term outcomes, including survival, presumably most notably in patients treated with curative intent.

Pegfilgrastim for prevention of febrile neutropenia

A randomized phase 3 double-blind, placebo-controlled clinical trial of primary prophylaxis with pegfilgrastim was conducted in patients with breast cancer receiving docetaxel 100 mg/m^2 every 3 weeks to determine the efficacy of pegfilgrastim when given with less myelosuppressive regimens. Patients were randomly assigned to pegfilgrastim 6 mg or placebo on the day following chemotherapy. Patients in the pegfilgrastim arm experienced significantly lower incidence of FN (1% vs. 17%), hospitalizations (1% vs. 14%) and anti-infective use (2% vs. 10%) (all $P < 0.001$). Pegfilgrastim is FDA approved to reduce the risk of FN in patients undergoing chemotherapy with a 17% or greater risk of FN without growth factor support (Table 4-3).

On the basis of the prolonged half-life of pegfilgrastim, it has been recommended that chemotherapy not be given sooner than 14 days after a dose of pegfilgrastim. Considerable experience with pegfilgrastim in support of every 2-week chemotherapy schedules, however, has demonstrated acceptable efficacy and safety. Otherwise, the safety profile of pegfilgrastim is similar to that of other forms of G-CSF.

GM-CSF for prevention of febrile neutropenia

GM-CSF is approved to reduce the risk of death from infections in patients ≥ 55 years old undergoing induction therapy for AML (Table 4-4). There is limited evidence from randomized trials for the use of GM-CSF in nonmyeloid malignancies, and it is not FDA approved for the prevention of FN in this population.

Clinical guidelines for the use of myeloid growth factors

The American Society of Clinical Oncology (ASCO), the National Comprehensive Cancer Network, and other organizations have developed guidelines for the use of

Table 4-5 American Society of Clinical Oncology guidelines for use of myeloid growth factors to prevent FN

Setting/indication	Recommended	Not recommended
General circumstances	FN risk in the range of 20% or higher	
Special circumstances	Clinical factors dictate use	
Secondary prophylaxis	Based on chemotherapy reaction among other factors	
Therapy of afebrile neutropenia		Routine use
Therapy of febrile neutropenia	If high-risk for complications or poor clinical outcomes	Routine use
AML	Following induction therapy, patients >55 years old most likely to benefit	Priming prior to cytotoxic chemotherapy outside a clinical trial
	After the completion of consolidation chemotherapy	
MDS		Routine use in neutropenic patients
Acute lymphocytic leukemia	After the completion of initial chemotherapy or first postremission course	
Radiotherapy	Consider if receiving radiation therapy alone and prolonged delays are expected	Patients receiving concurrent chemotherapy and radiation
Older patients	If ≥65 years old with diffuse aggressive NHL and treated with curative chemotherapy	
Pediatric population	Primary prophylaxis of pediatric patients with a likelihood of FN and the secondary prophylaxis or therapy for high-risk patients	G-CSF use in children with ALL

Source: Smith TJ, Khatcheressian J, Lyman GH, et al. *J Clin Oncol*. 2006;24:3187–3205.

NHL, non-Hodgkin lymphoma.

myeloid growth factors to prevent FN. In brief, current ASCO guidelines (Table 4-5) include the following:

1. Primary prophylaxis is recommended for patients at high risk (>20%) of FN due to age, medical history, disease characteristics, or the myelotoxicity of the chemotherapy regimen.
2. Primary prophylaxis should be given with “dose-dense” chemotherapy regimens.
3. Secondary prophylaxis after a neutropenia-related event has occurred generally is recommended if reduced dosing or dose intensity will compromise disease-free or overall survival or expected treatment outcome.

Specific factors predisposing to FN and serving as current indications to consider the use of myeloid growth factors are listed in Table 4-6.

Treatment for febrile neutropenia

All patients with FN should be treated empirically with antibiotics after a thorough physical examination directed at identifying a site of infection and after appropriate cultures are obtained. A number of studies have addressed whether patients with FN benefit from initiation of a myeloid

Table 4-6 Risk factors for chemotherapy-associated neutropenia and its complications

Age >65 years
Previous chemotherapy or radiation therapy
Bone marrow involvement of tumor
Preexisting neutropenia, infections, open wounds, or recent surgery
Poor performance status
Decreased renal function
Decreased liver function, particularly increased bilirubin level

Adapted from Crawford J, Armitage J, Balducci L, et al. *J Natl Comp Cancer Netw*. 2013;11:1266–1290.

growth factor in addition to broad-spectrum antibiotics. A meta-analysis of 13 randomized clinical trials compared the use of G-CSF or GM-CSF plus antibiotics with the use of antibiotics alone in patients with chemotherapy-induced FN. The meta-analysis showed that the use of a myeloid growth factor accelerated the time to neutrophil recovery and shortened hospital stay but did not affect overall survival. ASCO guidelines recommend that the myeloid growth factors should not be used routinely as adjuncts to antibiotics for patients with FN. These guidelines

recommend that the myeloid growth factors should be considered for patients expected to have prolonged (>10 days) and profound neutropenia ($<0.1 \times 10^9/L$); use also should be considered for those >65 years old with pneumonia, hypotension, invasive fungal infections, or sepsis.

Acute myeloid leukemia

Neutropenia, anemia, and thrombocytopenia are common presenting features of AML and also are important complications in its treatment. There are many studies of the use of myeloid growth factors to sensitize leukemic cells to increase the effectiveness of chemotherapy and prevent infectious complications. Although G-CSF and GM-CSF may shorten the duration of neutropenia during the induction phase of chemotherapy, neither consistently reduces the occurrence of FN, infections, or the duration of hospitalization. Results for sensitization of the leukemic cells to chemotherapy also are inconsistent, and use of the myeloid growth factors in this way is not recommended except for research studies.

During the consolidation phase of treatment, the marrow is more responsive, and 2 large randomized trials have demonstrated significant decreases in the duration of severe neutropenia with an associated decrease in infections requiring antibiotics with G-CSF therapy. No consistent favorable or detrimental impact of G-CSF or GM-CSF on treatment response and survival has been observed.

Acute lymphoblastic leukemia (ALL)

Neutropenia is a common consequence of treatment in patients with acute lymphoblastic leukemia (ALL). Eight randomized controlled trials, including more than 700 adults and children, demonstrated that neutrophil recovery is accelerated with myeloid growth factor therapy, mostly utilizing G-CSF. No consistent therapeutic benefits in reducing infections, shortening hospitalizations, or improving the overall treatment outcomes were observed.

Mobilization of autologous peripheral blood stem cells and enhancement of neutrophil recovery after autologous transplantation

Autologous peripheral blood stem cells are collected routinely from cancer patients by leukapheresis after cytoreductive chemotherapy or after cytoreductive chemotherapy followed by G-CSF or GM-CSF. Mobilization with G-CSF has been demonstrated to involve several steps. First, G-CSF markedly enhances neutrophil production. G-CSF administration also releases neutrophil elastase and cathepsin G from the granules of the developing marrow neutrophils. When released, these proteases cleave adhesion molecules expressed on the surfaces of the marrow

stromal cells. Cleavage of the bond of chemokine receptor 4 (CXCR4), expressed on hematopoietic progenitor cells, and its ligand chemokine ligand 12 (CXCL12, also known as stromal cell-derived factor 1 or SDF-1), expressed on marrow stromal cells, is thought to be the principal mechanism for progenitor cell release into the circulation.

As discussed in Chapters 13 and 14, transplantation of autologous peripheral blood stem cells results in the restoration of hematopoiesis after high-dose (myeloablative) chemotherapy. Clinical trials of autologous peripheral blood stem cell transplantation have shown that the use of a myeloid cytokine after stem cell infusion accelerates neutrophil recovery by 2 to 4 days. However, neutrophil recovery to $>0.5 \times 10^9/L$ is so rapid (median 11 to 14 days) without a myeloid growth factor that it has been difficult to demonstrate a meaningful clinical benefit of G-CSF or GM-CSF, including reduced risk of sepsis or death due to infection in patients receiving a peripheral blood stem cell product. Therefore, consensus on their use in this setting is lacking. A few randomized studies have found no difference in safety of pegfilgrastim as compared to filgrastim in this setting. Plerixafor, a CXCR4 antagonist, acts synergistically with G-CSF to yield greater numbers of CD34⁺ stem cells and is FDA approved as an adjunct to G-CSF for stem cell mobilization in certain conditions, particularly for patients who are expected to mobilize poorly with G-CSF alone.

Mobilization of peripheral blood stem cells from normal donors for allogeneic transplantation

G-CSF treatment of normal donors effectively mobilizes stem cells for use in subsequent allogeneic transplantation and has an excellent safety profile.

Acceleration of neutrophil recovery after bone marrow and umbilical cord blood transplantation

Peripheral blood stem cells are preferred over bone marrow in some instances because of the ease of collection of peripheral blood stem cells, lower risk of primary graft failure, and a more rapid neutrophil and platelet recovery. Nonetheless, bone marrow is preferred for many recipients as the risk of graft-versus-host disease is lower. When bone marrow transplantation is performed, a myeloid growth factor after bone marrow stem cell infusion significantly accelerates neutrophil recovery by approximately 4 to 5 days. A meta-analysis of 18 clinical trials totaling 1,198 patients showed no change in the risk of acute or chronic graft-versus-host disease after allogeneic stem cell transplantation with either GM-CSF or G-CSF when compared with patients who did not receive a myeloid growth factor.

Umbilical cord blood transplants have been able to extend the benefits of allogeneic transplant to those without a matched donor. As a result of the size and composition of the graft, hematopoietic recovery is prolonged, and recipients are at a higher risk for infectious complications. In retrospective studies, the use of G-CSF reduced the time to neutrophil recovery by approximately 10 days. Although prospective data are lacking, G-CSF is routinely used after cord blood transplant.

Improvement of neutrophil production in patients with delayed engraftment or graft failure after bone marrow transplantation

Patients who do not achieve a neutrophil count of $0.1 \times 10^9/L$ by day 21 after transplantation or whose neutrophil count drops below $0.5 \times 10^9/L$ following engraftment in the absence of relapse often respond to a myeloid growth factor with improvement in neutrophil production.

Severe chronic neutropenia (idiopathic, cyclic, congenital)

Severe chronic neutropenia is a heterogeneous group of inherited and acquired disorders characterized by a persistent neutrophil count of $<0.5 \times 10^9/L$ and recurrent bacterial infections, including Kostmann syndrome, sporadic and autosomal dominant severe congenital neutropenia, and cyclic neutropenia (see Chapter 15).

Most patients with congenital and cyclic neutropenia respond well to treatment with G-CSF. Treatment significantly improves neutrophil counts, dramatically decreases the incidence and severity of bacterial infections, and appears to improve survival. Responses can be maintained over many years with daily or alternate day G-CSF. Patients with cyclic neutropenia maintained on G-CSF continue to have regular fluctuations in the neutrophil count, but the depth of the nadir is reduced and lasts for fewer days. Patients with severe congenital neutropenia attributable to mutations in *ELANE*, *HAX1*, or *WAS* or as-yet-unknown mutations are at risk of developing AML. The lifetime risk is estimated to be as high as 30%. In contrast, there is no apparent risk of AML in patients with cyclic neutropenia.

The Severe Chronic Neutropenia International Registry is a useful source for additional information about the diagnosis and treatment of severe chronic neutropenia (<http://depts.washington.edu/registry/>).

Myelodysplasia

The myelodysplastic syndromes (MDS), also discussed in Chapter 17, are a group of acquired neoplastic hematopoietic stem cell disorders with the hallmark of ineffective

hematopoiesis. Both quantitative and qualitative defects in neutrophils impair the ability to ward off bacterial infection in these patients. A handful of clinical trials have investigated treatment of MDS with HGFs. Treatment with G-CSF or GM-CSF can normalize the neutrophil count in patients with MDS, but whether this translates into reduced mortality from bacterial or fungal infection is less clear. A randomized, phase 3 trial of 102 patients with high-risk MDS did not demonstrate a reduction in infectious complication but suggested an increase in nonleukemic disease-related deaths associated with the routine use of G-CSF to increase neutrophil counts. However, in low-risk MDS, G-CSF or GM-CSF may enhance the effects of erythropoietin in the treatment of MDS-related anemia. There is no convincing evidence at present that growth factor therapy accelerates progression from low-risk MDS to AML.

Other potential clinical uses of G-CSF

HIV

Neutropenia is common in HIV infection and is found in 5% to 10% and 50% to 70% of patients with early and advanced disease, respectively. Furthermore, medications used in the management of HIV, associated opportunistic infections, and malignancies can lead to neutropenia. Treatment with G-CSF promptly increases the neutrophil count to the normal range in most patients. A large multicenter trial that randomized 258 HIV-positive patients with a low CD4 count ($0.2 \times 10^9/L$) and absolute neutrophil count of 0.75×10^9 to $1.0 \times 10^9/L$ showed that G-CSF-treated patients (dose adjusted to increase the absolute neutrophil count to 2.0×10^9 to $10.0 \times 10^9/L$) had fewer bacterial infections, less antibiotic use, and fewer hospital days, but no change in viral load, in comparison with the control group.

Leukapheresis

Large numbers of neutrophils can be collected by leukapheresis from normal donors pretreated with G-CSF plus dexamethasone, and these neutrophils exhibit normal function in vitro. Transfusion of G-CSF-stimulated neutrophil leukapheresis products into severely neutropenic leukemia patients or stem cell transplant recipients can transiently raise the peripheral neutrophil count to the normal range ($<2.0 \times 10^9/L$). Whether neutrophil transfusions increase survival in patients with profound sustained neutropenia who have an active bacterial or fungal infection is under investigation.

Diabetic foot infections

The role of myeloid growth factors for the treatment of diabetic foot infections is unclear. A meta-analysis

summarized the potential benefits of G-CSF as an adjunctive therapy. On the basis of an analysis of 5 trials with a total of 167 patients, this review showed that G-CSF did not significantly affect the likelihood of resolution of the infection or wound healing, but its use was associated with significantly reduced likelihood of lower extremity surgical interventions, including amputation. G-CSF treatment appears to reduce the duration of hospital stay but not the duration of systemic antibiotic treatment. The evidence suggests benefit, but it is unclear exactly which patients may be helped by adjunctive G-CSF.

Pneumonia

A number of clinical trials have explored the use of G-CSF in non-neutropenic adults with community-acquired pneumonia or hospital-acquired pneumonia. In an evidence-based review, 6 studies with a total of 1,984 people were identified. G-CSF use appeared to be safe, with no increase in the incidence of serious adverse events. The use of G-CSF, however, was not associated with improvement in mortality at 28 days.

Myocardial infarction

Studies have suggested that stem cells mobilized from the marrow by myeloid growth factor may improve cardiac function following myocardial infarction, presumably by stimulating angiogenesis. However, a recent meta-analysis of 7 trials involving 354 patients who received myeloid growth factor or placebo for 4 to 6 days after acute myocardial infarction found no difference in mortality and no improvement in parameters of left ventricular function.

In 1 small prospective clinical study, G-CSF therapy with intracoronary infusion of peripheral blood stem cells showed improved cardiac function and promoted angiogenesis in patients with myocardial infarction. Aggravation of in-stent restenosis led to early termination of the study. Although studies such as these are intriguing for the utilization of G-CSF-mobilized stem cells for a variety of new applications, no conclusive evidence exists at present supporting these applications.

Side effects of G-CSF

The major side effect of G-CSF is bone pain in the hips, which usually coincides with marrow recovery and may be due to the expansion of hematopoiesis within the marrow cavity. Medullary bone pain occurs in approximately 30% of patients treated with G-CSF, and osteoporosis has been observed in some patients who were administered G-CSF. Other side effects of G-CSF include headache and fatigue. G-CSF should not be used in patients with sickle cell disease; case reports document the precipitation

of sickling and severe pain crisis in these individuals. Other rare side effects include splenic rupture and adult respiratory distress syndrome.

Side effects of GM-CSF

The major side effect of GM-CSF is a flu-like illness characterized by fever (22% of patients) and myalgias and arthralgias (15%). A fraction of patients treated with GM-CSF experience fluid retention (8%) or dyspnea (13%). GM-CSF should not be used concurrently with chemotherapy. A case report detailed the abrupt onset of sickle cell pain crisis in a patient who received GM-CSF injections around a chronic leg ulcer.

Risk of leukemia with G-CSF and GM-CSF

Concerns have been expressed that G-CSF and GM-CSF might cause leukemia as they are known to stimulate proliferation of leukemic blasts. At present there is no convincing evidence that treatment outcomes for AML are worsened by myeloid growth factor treatments used in conjunction with appropriate chemotherapy. In patients receiving myelotoxic chemotherapy agents for other types of cancer, there is a significant risk of secondary leukemias. This risk probably is related directly to specific leukemogenic chemotherapy agents and regimens. Recent analysis of data from randomized trials suggests that the risk of AML may be increased in those receiving chemotherapy supported by the myeloid growth factors, but interpretation of the results is made difficult by the observation that myeloid growth factor-treated patients usually receive larger doses and longer courses of chemotherapy. The long-term risk of leukemia is also of importance to normal stem cell donors, but little information exists regarding donors mobilized with myeloid growth factors. It is estimated that it will require the observation of a little over 2,000 donors for a minimum of 10 years to detect a tenfold increase in the incidence of leukemia. However, it is important to note that patients with idiopathic or cyclic neutropenia have received G-CSF for many years without progression to leukemia.

New formulations of G-CSF and GM-CSF

Because of the potency and effectiveness of G-CSF and GM-CSF, there have been many efforts to identify additional myeloid growth factors and make new derivatives from the parent molecules. Several new products with a prolonged duration of their stimulatory effects, similar to pegylated G-CSF, are in development. A key issue is whether or not the new molecules are immunogenic. The development of antibodies to a growth factor can be hazardous, as they can block the activity of the adminis-

tered drug and also neutralize the effects of the naturally produced, endogenous growth factors, thus worsening neutropenia.

The number of laboratories and biopharmaceutical companies producing myeloid growth factors is also rapidly increasing. Their products are molecularly similar to the approved products and are called “biosimilars.” Testing and introduction of biosimilars is proceeding rapidly with the first application for a biosimilar filgrastim being accepted by the FDA in mid-2014. As of the time of writing, the recently approved tbo-filgrastim is not technically considered a filgrastim biosimilar because it was filed as a biologics license application, since a biosimilars approval pathway had not been established at the time of FDA submission.

Erythroid growth factors

Erythropoietin

EPO is the principal HGF that regulates red blood cell production. The liver is the primary site of EPO production during fetal development. In adults, EPO is produced predominantly in the kidney, with a small amount produced in the liver. Renal EPO production is under the control of an oxygen-sensing mechanism involving transcriptional regulation by hypoxia-inducible factor (HIF). HIF signaling and local EPO production in osteoblasts in the hematopoietic stem cell niche have been reported. Plasma EPO levels are measurable by a clinically available enzyme-linked immunosorbent assay. In some patients with nonrenal anemia, the degree of plasma EPO elevation may assist in predicting response likelihood to recombinant human EPO (rhEPO) therapy.

EPO exerts its erythropoietic action by binding to its specific high-affinity cell surface receptor (EPOR) expressed on erythroid progenitor and precursor cells in the bone marrow (see video in online edition). EPOR does not possess intrinsic tyrosine kinase enzymatic activity. Its intracellular domain associates with a cytoplasmic tyrosine kinase, Janus kinase 2 (JAK2), to activate downstream signaling that promotes the proliferation, survival, and differentiation of erythroid cells. Low levels of EPOR expression have been found in neural tissues, endothelial cells, and other nonhematopoietic cell types. Targeted disruption of the genes encoding either EPO or EPOR in mice leads to severe in-utero anemia and embryonic death. Cardiovascular and neural abnormalities also have been reported. These mice exhibit normal formation of early and late erythroid progenitors, burst-forming unit–erythroid and colony-forming unit–erythroid, indicating

that commitment to erythroid lineage does not require EPO but rather that terminal differentiation of colony-forming unit–erythroid into mature red blood cells depends on intact EPOR signaling.

Naturally occurring, dominant gain-of-function EPOR gene mutations that disrupt downregulation of JAK2 activation are associated with primary familial and congenital polycythemia. An acquired, somatic, activating *JAK2* V617F mutation is encountered in 95% of polycythemia vera cases and in about 50% of patients with other BCR-ABL1-negative myeloproliferative neoplasms. Mutations in the genes encoding HIF, von Hippel-Lindau proteins, and prolyl hydroxylase domain (PHD) enzymes that regulate renal oxygen sensing and EPO production are found in some patients with secondary familial and congenital polycythemia due to inappropriate elevation of plasma EPO levels.

Recombinant human erythropoietins

Three main rhEPO preparations—epoetin alfa, epoetin beta, and darbepoetin alfa—are available for clinical use in the United States and Europe. The biologic activity and adverse effect profiles of these agents are comparable. The difference in the amount of posttranslational glycosylation of each product modulates the pharmacokinetic properties. These agents are produced by recombinant DNA technology, by a mammalian cell line into which the *EPO* gene has been introduced. Biosimilar products (“follow-on biologics”) for epoetins have been available in some countries as the patent and exclusivity rights have expired.

Epoetin alfa was the first recombinant product approved by the FDA in 1989 for its indication in chronic kidney disease (CKD) patients, followed by its approval in 1993 in the oncology supportive care setting for chemotherapy-induced anemia (Table 4-6).

Epoetin beta is available for clinical use in Europe. Darbepoetin alfa is a hyperglycosylated form and binds to the same cellular receptor. The modification of 2 additional N-linked oligosaccharide chains compared with EPO leads to a higher molecular weight than EPO and a threefold longer half-life in vivo. The advantage is that it can be administered less frequently than epoetin alfa or epoetin beta to achieve a comparable increment in hemoglobin. Darbepoetin alfa was approved by the FDA for clinical use in 2001 (Table 4-7).

Continuous erythropoietin receptor activator (CERA) is a structurally distinct pegylated epoetin beta product containing a methoxy polyethylene glycol polymer. This modification extends its half-life, allowing the dosing intervals to be prolonged up to once every 4 weeks to maintain hemoglobin levels in CKD patients on dialysis. CERA is approved for use in some European countries, as well as

Table 4-7 FDA-approved indications for epoetin alfa

Anemia due to:
Chronic kidney disease in patients on dialysis and not on dialysis
The effects of concomitant myelosuppressive chemotherapy, and upon initiation, there is a minimum of an additional 2 months of planned chemotherapy
Zidovudine in HIV-infected patients, and for reduction of allogeneic red cell transfusions in patients undergoing elective, noncardiac, nonvascular surgery

by the FDA. A long-acting CERA, called Mircera, is used commonly to treat the anemia associated with CKD and in dialysis patients.

FDA-approved clinical uses of rhEPO

Chronic kidney disease

Normocytic, normochromic anemia associated with EPO deficiency occurs in the majority of patients with CKD during progression to end-stage renal disease. Anemia contributes to CKD-related symptoms and has been associated with the presence of cardiac comorbidities, reduced quality of life, and increased risk of mortality. In patients with anemia due to CKD, rhEPO therapy improves hemoglobin levels and eliminates transfusion requirements; however, studies have shown that targeting and maintaining near-normal or normal hemoglobin levels is associated with increased morbidity and mortality risk.

Following a safety review in 2011, the FDA mandated changes to the drug labels for epoetin alfa and darbepoetin alfa, warning that in controlled trials patients experienced greater risks of death, serious adverse cardiovascular reactions, and stroke when they were administered rhEPO to target a hemoglobin level >11 g/dL. It was noted that no trial has identified a hemoglobin target level, rhEPO dose, or dosing strategy that does not increase these risks.

Effective 24 June 2011, the FDA safety announcement indicated the following:

- Consider starting rhEPO treatment when hemoglobin level is <10 g/dL, without specifying how far below 10 g/dL is appropriate for an individual to initiate therapy. It is recommended to individualize dosing and use the lowest dose sufficient to reduce the need for red blood cell transfusions. A target hemoglobin level is not specified.
- For patients with CKD not on dialysis, consider initiating rhEPO treatment only when hemoglobin level is <10 g/dL and if the rate of hemoglobin decline indicates the likelihood of requiring a red blood cell transfusion and reducing alloimmunization or other

transfusion-related risks is a goal. If the hemoglobin level exceeds 10 g/dL, reduce or interrupt rhEPO dose and use the lowest dose sufficient to reduce the need for transfusions.

- For patients with CKD on dialysis, initiate rhEPO treatment when hemoglobin is <10 g/dL. If the hemoglobin level approaches or exceeds 11 g/dL, reduce or interrupt the dose of rhEPO.
- For patients who do not respond adequately over a 12-week escalation period, increasing the rhEPO dose further is unlikely to improve response and may increase risks.

The initial dose of epoetin alfa in predialysis CKD patients is typically 50 to 100 U/kg administered subcutaneously once a week. Most patients respond to a regimen of 10,000 U/week. Darbepoetin alfa 60 mg every 2 weeks subcutaneously is an alternative regimen in predialysis patients.

For hemodialysis patients, the recommended initial dose of epoetin alfa is 50 to 100 U/kg 3 times per week. The weekly epoetin dose requirement was shown to be about 30% less with subcutaneous administration as compared with intravenous route in a randomized trial involving patients on hemodialysis. Most hemodialysis patients, however, receive epoetin alfa intravenously because of discomfort with subcutaneous injections and the convenience of an intravenous route during dialysis. Darbepoetin alfa typically is started at 0.45 mg/kg administered intravenously once a week.

Before and during rhEPO therapy, iron stores are assessed and monitored to avoid development of iron deficiency and to achieve maximum benefit from rhEPO. Ferritin levels typically are maintained at ≥ 100 ng/dL and the transferrin saturation at 20%. Many hemodialysis patients require intravenous iron infusions to ensure the adequacy of iron stores during rhEPO therapy.

Cancer patients receiving myelosuppressive chemotherapy

Patients with nonmyeloid malignancies receiving myelosuppressive chemotherapy frequently develop mild to moderate anemia. To ameliorate cancer- or chemotherapy-induced anemia and its associated symptoms such as fatigue, about 50% of patients require red blood cell transfusions during the course of their illness. In this clinical setting, epoetin alfa and darbepoetin alfa exhibit efficacy in increasing hemoglobin and reducing the requirement for red blood cell transfusions during chemotherapy. In a series of 9 meta-analyses, the relative risk for transfusion ranged from 0.58 to 0.67 in rhEPO-treated patients. Although the risks associated

with allogeneic transfusions are avoided in some patients treated with rhEPO, the requirement for transfusions is not completely eliminated.

Several clinical trials and meta-analyses have reported that rhEPO therapy for chemotherapy-induced anemia may improve quality of life as measured by Functional Assessment of Cancer Therapy instruments. More recently, the presence, magnitude, and clinical significance of any potential beneficial effect of rhEPO on quality of life has been controversial, especially in the context of the accumulating evidence of risks of rhEPO therapy in this patient population, leading to use restrictions to minimize the potential for harm.

In 2008, the FDA mandated changes to the labels of epoetin alfa and darbepoetin alfa based on risks of shortened survival or increased risk of tumor progression in cancer patients, as well as the risks of cardiovascular complications reported in other studies. Starting in 2010, prescribers and hospitals had to enroll in and comply with the REMS (risk evaluation and mitigation strategy) program, termed the ESA APPRISE Oncology Program (*Assisting Providers and Cancer Patients with Risk Information for the Safe Use of Erythropoiesis-Stimulating Agents*) to prescribe or dispense rhEPO products to patients with cancer. Over the ensuing 5 years, the use of erythroid stimulating agents, especially in the setting of chemotherapy-induced anemia, dropped substantially. The REMS program stopped in 2017. The FDA-approved label for rhEPOs currently recommends the following:

- Use the lowest dose needed to avoid red blood cell transfusions.
- Use rhEPO only for anemia from myelosuppressive chemotherapy.
- rhEPO is not indicated for patients receiving myelosuppressive chemotherapy when the anticipated outcome is cure. The specific types of malignancies were not indicated.
- Initiate rhEPO only if hemoglobin is <10 g/dL, and if there is a minimum of an additional 2 months of planned chemotherapy.
- Reduce dose by 25% if hemoglobin increases >1 g/dL in any 2-week period or if hemoglobin reaches a level at which transfusion is not required.
- Withhold dose if hemoglobin exceeds a level needed to avoid red cell transfusion.
- Discontinue use if there is no hemoglobin response or if transfusions are still required after 8 weeks of therapy.
- Discontinue following the completion of a chemotherapy course.

Table 4-8 FDA-approved indications for darbepoetin alfa

Anemia due to:
Chronic kidney disease in patients on dialysis and patients not on dialysis
The effects of concomitant myelosuppressive chemotherapy, and upon initiation, there is a minimum of an additional 2 months of planned chemotherapy

Table 4-9 FDA-approved indications for romiplostim and eltrombopag

Thrombocytopenia due to:
Chronic ITP in adults with an insufficient response to corticosteroids, immunoglobulins, or splenectomy (romiplostim and eltrombopag); and in adults and children as young as 1 year old (eltrombopag only), though studies of romiplostim in pediatric patients with ITP have been completed.
Chronic hepatitis C to allow the initiation and maintenance of interferon-based therapy (eltrombopag only)
Severe aplastic anemia with an insufficient response to immunosuppressive therapy (eltrombopag only)

ITP, immune thrombocytopenia.

The typical starting dose of epoetin alfa is 150 U/kg subcutaneously 3 times per week, or 40,000 U subcutaneously weekly until completion of a chemotherapy course. The starting dose for darbepoetin alfa is 2.25 µg/kg/week or 500 µg every 3 weeks subcutaneously until completion of a chemotherapy course. An alternative darbepoetin regimen is 200 µg every 2 weeks with comparable efficacy to epoetin alfa 40,000 U weekly. Hemoglobin level is monitored weekly until stable. Previous studies have not identified a specific plasma endogenous EPO level above which patients would be less likely to respond to rhEPO therapy, though the higher the baseline EPO level, the less likely there will be a response to exogenous EPO.

Iron stores should be assessed before initiation of therapy and monitored periodically during therapy. Oral or parenteral iron supplementation may be required in some patients to maximize response to rhEPO. In patients who fail to respond to rhEPO, considerations include concomitant iron deficiency, blood loss, vitamin deficiencies (B_{12} and folate), hemolysis, anemia associated with the malignancy (“anemia of cancer”), or an underlying hematologic disorder.

American Society of Hematology/American Society of Clinical Oncology clinical practice guidelines

The American Society of Hematology (ASH)/American Society of Clinical Oncology (ASCO) Update Committee reviewed data published between January 2007 and January

2010 and presented the following recommendations for clinicians treating patients undergoing myelosuppressive chemotherapy who have a hemoglobin level <10 g/dL:

- Identify alternative causes of anemia aside from chemotherapy or underlying hematologic malignancy.
- Clinicians are advised to discuss potential harms (eg, thromboembolism, shorter survival) and benefits (eg, decreased transfusions) of rhEPO therapy compared with potential harms (eg, serious infections and immune-mediated adverse reactions) and benefits (eg, rapid hemoglobin improvement) of red blood cell transfusions.
- If used, rhEPO should increase hemoglobin to the lowest concentration possible to avoid transfusions and it should be administered at the lowest dose possible.
- Available evidence does not identify hemoglobin levels ≥ 10 g/dL either as a threshold for initiating treatment or as targets for rhEPO therapy.
- Starting doses and dose modifications should follow FDA-approved labeling.
- rhEPO should be discontinued after 6 to 8 weeks in nonresponders.
- rhEPO should be avoided in cancer patients not receiving concurrent chemotherapy, except for those with lower risk MDS.
- Caution is recommended when using rhEPO with chemotherapeutic agents in diseases associated with increased risk of thromboembolic complications.

Anemia associated with HIV infection

The prevalence and severity of anemia in patients with HIV disease have decreased in the era of highly active antiretroviral therapy. In a cohort of 9,690 patients, anemia (hemoglobin <14 g/dL in men; <12 g/dL in women) was observed in 36%. More severe anemia (hemoglobin <11 g/dL in men; <10 g/dL in women) was infrequent, observed in 5% of patients.

The pathogenesis of HIV-related anemia is often complex and multifactorial, including myelosuppressive effects of various drugs (notably zidovudine, co-trimoxazole, and ganciclovir); coinfections; inflammation causing iron utilization defect; HIV infection of marrow stromal cells, which limits their ability to support erythropoiesis; and mild relative EPO deficiency in some patients. Bleeding, autoimmune or drug-induced hemolysis, iron or folate deficiency also may contribute. Risk factors for anemia development include zidovudine use, CD4 lymphocyte count $<0.2 \times 10^9/L$, high HIV viral load, African American ethnicity, and female sex.

Anemia in HIV infection is independently associated with decreased survival, and retrospective analyses suggest that recovery from anemia is associated with decreased risk of death. Although rhEPO therapy has been reported to increase hemoglobin level and reduce transfusions in some patients, there is no evidence that survival is improved as a result of rhEPO therapy.

In early studies, epoetin alfa (100 to 200 U/kg 3 times per week) was reported to significantly improve hemoglobin levels and reduce transfusion requirements in patients with AIDS who were receiving zidovudine, with endogenous plasma EPO level <500 U/L. Epoetin alfa given once per week (40,000 to 60,000 U) for patients with hemoglobin <12 g/dL was reported to be effective in raising hemoglobin level and improving quality of life. Previous studies have not addressed the issue of optimal target hemoglobin in this clinical setting. Caution is advisable given the reported adverse effect profile in CKD and cancer patients associated with targeting normal hemoglobin levels. In the HIV disease setting, the current FDA-approved label indicates to dose epoetin alfa to achieve a hemoglobin level needed to avoid red blood cell transfusions, to withhold therapy if hemoglobin exceeds 12 g/dL, and to discontinue therapy if no increase in hemoglobin is observed at 8 weeks at a dose level of 300 U/kg per week.

Allogeneic blood transfusions in patients undergoing surgery

Perioperative epoetin alfa administration reduces the risk of allogeneic blood transfusions in patients undergoing major elective, nonvascular, noncardiac surgery, primarily studied in the orthopedic surgery setting. The FDA-approved regimens for this indication are 300 U/kg daily subcutaneously for 14 days total, administered daily for 10 days before surgery, on the day of surgery, and for 4 days after surgery. In patients undergoing major orthopedic surgery with pretreatment hemoglobin of 10 to 13 g/dL, significantly fewer epoetin-treated patients (23%) required transfusions compared with a placebo group (45%). In the cohort with baseline hemoglobin of 13 to 15 g/dL, there was no significant difference in the number of patients transfused (9% for epoetin alfa and 13% for placebo). An alternative approved epoetin alfa regimen is 600 U/kg/week subcutaneously administered 21, 14, and 7 days before surgery and on the day of surgery. Consideration of antithrombotic prophylaxis is recommended during perioperative epoetin alfa therapy.

Two modified epoetin alfa regimens were investigated in a randomized, double-blind, placebo-controlled trial involving 201 patients undergoing primary hip arthroplasty

with hemoglobin level 9.8 to 13.7 g/dL. Four weekly doses (20,000 or 40,000 U) starting 4 weeks before surgery were administered along with oral iron supplementation. Both epoetin alfa regimens significantly reduced the requirement for allogeneic blood transfusions (22.8% for the low-dose and 11.4% for the high-dose group) compared with the placebo group (44.9%). The incidence of thromboembolic events was not different between groups.

In a trial of 680 patients undergoing spinal surgery who did not receive thromboprophylaxis, patients were randomized to preoperative epoetin alfa 600 U/kg for 4 doses (21, 14, and 7 days prior to surgery and on the day of surgery) or standard care. There was an increased incidence of deep vein thrombosis (4.7%) in the epoetin alfa-treated cohort compared with the standard care patient group (2.1%).

Preoperative epoetin alfa treatment has been used to facilitate autologous blood donation, although routine application for this indication is not justified in clinical practice for reasons of cost and safety; notably, an increased risk of postoperative venous thromboembolism if hemoglobin levels are elevated at the time of surgery. Selected anemic patients who are willing to donate autologous blood or those who decline allogeneic or autologous red blood cell transfusions based on their religious beliefs may benefit from preoperative epoetin therapy. One study randomized patients with mild anemia (hematocrit $\leq 39\%$) to treatment with 3 different dosing regimens of epoetin alfa or placebo beginning 25 to 35 days before surgery. Iron supplementation was given intravenously. A dose-dependent increase in the number of autologous units donated was observed.

Other clinical uses of rhEPO

Anemia in patients declining transfusion

The published literature is dotted with small series and case reports discussing the use of erythropoiesis-stimulating agents in patients who decline allogeneic or autologous blood transfusion. One such report reviewed the outcomes of 500 Jehovah's Witness patients undergoing cardiac surgery at a single center. This study compared an evolving bloodless surgical strategy in 2 successive eras. In addition to blood-conserving operative techniques, the backbone of this regimen was the administration of epoetin alpha 300 U/kg intravenously, plus 500 U/kg subcutaneously, on admission. After surgery, 500 U/kg was given subcutaneously every second day, along with oral iron supplementation. Aminocaproic acid was also given from the time of anesthesia induction to skin closure. For the patients managed with this strategy, the 30-day mortality from the time

of surgery ranged from 1% to 3%. Data on thrombotic events was not reported. In light of the risk of venous thromboembolism associated with use of erythropoiesis-stimulating agents in patients with a hemoglobin level over 10 g/dL, as per the FDA's black box warning, it is difficult to reconcile the potential risks and benefits of this approach. The ongoing Transfusion Indication Threshold Reduction 2 (TITR-e2, NCT0923932) randomized trial is expected to provide insight into what is an acceptable transfusion threshold in patients undergoing cardiac surgery, the results of which will be directly applicable to the care of Jehovah's Witness and other patients who decline transfusions.

Anemia in preterm infants

Anemia of prematurity in very-low-birth-weight ($< 1,500$ g) infants born before the third trimester of pregnancy is associated with multiple factors, including rapid infant growth and expansion of blood volume, shortened life span of neonatal red blood cells, and inadequate EPO production in response to anemia. Iatrogenic factors, such as phlebotomies for laboratory tests during critical illness, exacerbate the problem. Many infants require red cell transfusions for symptomatic anemia.

The physiologic decrease in circulating red cells that occurs during the first weeks of life in all neonates is more pronounced and rapid in low-birth-weight preterm infants. The switch of the primary site of EPO production from the liver to the kidney, which normally occurs after birth, has not taken place in the preterm infant. EPO production in the liver is less sensitive to anemia and hypoxia, leading to relatively diminished EPO synthesis.

Although rhEPO therapy has been reported to reduce red blood cell transfusions in very-low-birth-weight infants, questions remain regarding the clinical significance of this beneficial effect in terms of the absolute reduction in transfusion volume achieved and whether exposure to multiple blood donors and alloimmunization risk is prevented by rhEPO therapy. Furthermore, the implementation of stringent transfusion criteria in clinical practice has reduced the number and volume of transfusions independent of rhEPO. For these reasons, rhEPO therapy in the setting of anemia of prematurity is not widely adapted into routine clinical practice.

Retrospective data from a few studies and a meta-analysis suggested a link between rhEPO therapy and exacerbation of retinopathy of prematurity, a disorder of vascular proliferation. At present, no conclusive data demonstrate a direct role for rhEPO in retinopathy of prematurity. The possibility of a link, however, raises concerns in view of the reported association between endogenous EPO and

pathologic neovascularization of proliferative diabetic retinopathy in adults.

Myelodysplastic syndromes

Anemia is the most common cytopenia encountered in patients with MDS. rhEPO has been used as monotherapy or in combination with G-CSF for treatment of anemia in MDS. Studies using darbepoetin alfa report erythroid response rates that are comparable to those with epoetin alfa or beta. These drugs do not carry an FDA-approved indication for anemia associated with MDS.

The erythroid response rate, reported in single-arm studies, varies widely between 20% and 50% depending on patient selection and the response criteria used. Factors predicting better response rate to therapy include a low transfusion requirement (<2 units/month), low endogenous pretreatment plasma EPO level (<500 U/L), <10% bone marrow blasts, and low/intermediate-1 (int-1) risk International Prognostic Scoring System (IPSS). The addition of low-dose G-CSF may augment the hemoglobin response to rhEPO therapy, although the role of G-CSF therapy on the biology and course of MDS has not been defined. Meta-analyses have suggested that higher weekly epoetin or darbepoetin doses may elicit better erythroid response rate; however, the optimal doses of these agents have not been studied in prospective, randomized studies. Therapy typically is maintained for 12 weeks to assess efficacy and then should be continued until the positive effect on anemia and transfusion requirements is lost.

No randomized study to date has shown definitively that rhEPO therapy affects the natural course of patients with MDS. A small, prospective randomized trial compared supportive care alone to epoetin alfa (with or without G-CSF) in anemic patients with lower-risk MDS. Epoetin alfa was administered at a daily dose of 150 U/kg. At 4 months, the erythroid response rate was 36% in the epoetin group compared with 9.6% for supportive care. The secondary objectives, including quality of life measures and overall survival, were significantly better in epoetin responders compared to nonresponders. AML transformation was not different between the groups.

Two retrospective studies have reported improved survival in responders to rhEPO therapy compared with nonresponders. The largest retrospective study involved 403 patients with de novo MDS (303 patients IPSS low and int-1 risk). The epoetin alfa or beta regimen was 60,000 U weekly, and darbepoetin alfa was 300 µg weekly for at least 12 weeks. Some patients (33%) also received G-CSF. The erythroid response rate was 40% or 50% using different response assessment criteria. Median duration of response was 20 weeks from the onset of rhEPO therapy. Compared with

a historical, untreated MDS cohort, rates of AML progression were similar. Overall survival was better in rhEPO responders compared with nonresponders or compared with untreated, matched, historical controls.

Investigational uses of rhEPO

rhEPO was shown to exert neuroprotective and cardioprotective effects in preclinical experimental models of tissue injury and in clinical pilot studies. These findings constituted the rationale for randomized, placebo-controlled clinical trials designed to investigate the safety and efficacy of rhEPO to improve outcomes in patients with acute stroke and coronary syndromes. In a clinical trial of patients with acute ischemic stroke, however, rhEPO treatment was not associated with an improvement in clinical recovery. There was a higher death rate in rhEPO-treated patients as compared with patients receiving placebo, particularly in those who were treated with thrombolysis.

In a series of randomized, placebo-controlled clinical trials involving patients with ST-segment elevation myocardial infarction undergoing percutaneous coronary intervention, rhEPO treatment did not reduce infarct size or improve left ventricular ejection fraction. Higher rates of adverse cardiovascular events, particularly in older patients, were reported in some studies.

The safety and efficacy of rhEPO in reducing allogeneic transfusions have been investigated in the intensive care setting in patients with or without trauma. In randomized trials, the effect of rhEPO on red blood cell transfusion requirements was inconsistent. In a trial involving 1,460 patients, epoetin alfa did not reduce the frequency of red blood cell transfusions. There was a significant increase in thrombotic events. There was a suggestion of reduced mortality in the subset of trauma patients; however, this outcome requires additional clinical investigation.

The prevalence of anemia in patients with congestive heart failure ranges from 15% to 50%. The etiology is thought to be multifactorial, including hemodilution, inflammation, renal dysfunction, iron deficiency, and use of angiotensin-converting enzyme inhibitors. Anemia in patients with heart failure is consistently associated with worse symptoms, functional impairment, and higher risk of death compared with nonanemic patients. A series of small clinical trials of rhEPO therapy reported increased hemoglobin levels associated with improved exercise capacity and left ventricular ejection fraction. However, in 2013, the RED-HF trial (2,278 subjects randomized to darbepoetin vs. placebo) demonstrated that treatment with darbepoetin did not improve clinical outcomes in patients with systolic heart failure and mild-to-moderate anemia.

Adverse effects associated with rhEPO therapy

The safety profile and adverse effects of epoetins and darbepoetin alfa are considered to be comparable. Cardiovascular adverse effects, venous thromboembolism, and increased mortality or tumor progression in cancer patients constitute the major concerns. Pure red cell aplasia due to the development of anti-EPO antibodies is rare and has been described predominantly in patients with CKD.

Cardiovascular adverse effects

rhEPO use may be associated with exacerbation of hypertension, particularly in patients with CKD, and therefore therapy should not be initiated in individuals with uncontrolled hypertension. Blood pressure monitoring is essential and avoiding rapid rise of hemoglobin during therapy may ameliorate the risk of hypertension. An increase of blood pressure medication dose may be required during rhEPO therapy. Hypertensive encephalopathy may be associated with a rapid rise in blood pressure. Seizures, usually related to uncontrolled hypertension, rarely may occur.

A series of randomized clinical trials raised concern for worse cardiovascular outcomes and mortality in CKD patients treated with rhEPO to achieve and maintain normal or near-normal hemoglobin levels compared with lower levels. The Normal Hematocrit Trial randomized 1,233 hemodialysis patients with cardiac disease to epoetin alfa therapy to achieve a hematocrit target of 30% or 42%. There was an insignificant trend toward an increase in nonfatal myocardial infarcts or death associated with increased hematocrit, leading to early termination of the study.

In predialysis CKD patients, the CHOIR study involved 1,432 epoetin alfa–treated patients randomized to target a hemoglobin of 13.5 g/dL or 11.3 g/dL. This study was terminated early due to a significant (34%) increase in composite cardiovascular outcome (death, myocardial infarction, hospitalization for congestive heart failure or stroke) in the normal hemoglobin group. Post hoc analyses suggested that failure to achieve the target hemoglobin and a requirement for higher doses of epoetin alfa were associated with increased risk of adverse cardiovascular outcomes.

The TREAT trial randomized 4,038 predialysis CKD patients with diabetes and anemia to treatment with darbepoetin alfa, either to a hemoglobin target of 13 g/dL or to placebo with matching rescue darbepoetin when hemoglobin was <9 g/dL. There was a doubling of the risk of stroke in patients assigned to darbepoetin compared with placebo. It is noteworthy that in the subset of patients with a history of cancer at baseline, significantly more patients

died of cancer in the darbepoetin group compared with placebo. In a follow-up analysis of the TREAT trial data, a poor initial response to darbepoetin was associated with an increased subsequent risk of death or cardiovascular events, as doses were escalated to meet target hemoglobin levels.

Venous thromboembolism

In the supportive oncology setting, rhEPO therapy is associated with increased venous thromboembolism risk, observed in both literature-based and individual patient data meta-analyses as well as in randomized controlled trials. The overall rate of these events is relatively infrequent. For instance, a literature-based meta-analysis reported venous thromboembolism in 7.5% of 4,610 patients treated with rhEPO compared with 4.9% of 3,562 control patients (relative risk, 1.57; 95% confidence interval [CI], 1.31–1.87). The mechanisms of venous thromboembolic events are not well defined and a conclusive link between hemoglobin levels and increased thromboembolism risk has not been established. Increased risk of arteriovenous access thrombosis in hemodialysis patients has been reported in association with higher hemoglobin levels.

Mortality or tumor progression in cancer patients

A series of clinical trials since 2003 reported adverse effects, including tumor progression or increased mortality in some rhEPO-treated patients, across a diverse group of malignancies—including lymphoproliferative malignancies and head-neck, breast, non-small-cell lung, uterine cervix, and mixed nonmyeloid cancers. The safety signals in these trials led to implementation of rhEPO use restrictions and REMS to minimize the potential for harm. Four of the 8 trials involved chemotherapy-treated patients, 2 trials included patients treated with radiotherapy only, and 2 trials involved patients with advanced cancer who did not receive antitumor therapy. In all 8 trials, the target hemoglobin level during rhEPO treatment was >12 g/dL, higher than presently recommended. In 2 trials, however, the achieved hemoglobin level was <12 g/dL, therefore raising concern that adverse rhEPO effects may occur at lower hemoglobin levels as well.

An individual patient data meta-analysis evaluating the effect of rhEPO therapy on mortality risk and survival included 53 studies with 13,933 patients. There was a significantly increased mortality risk (hazard ratio: 1.17, 95% CI 1.06–1.30, $P=0.003$) during the active study period associated with rhEPO therapy. In the subgroup of patients receiving chemotherapy, the observed increase in mortality risk did not reach statistical significance (hazard ratio: 1.10, 95% CI 0.98–1.24, $P=0.12$). In this meta-analysis, it was not possible to conclusively identify a subgroup of patients

with either an increased or decreased mortality risk when receiving rhEPO compared with other patients. rhEPO dosing frequency three or more times a week compared with less frequent schedules (once a week or once every 2 weeks) was associated with reduced mortality, although there were confounding factors in this analysis and a dose-response association was not detected.

Pure red cell aplasia

Pure red cell aplasia is a rare complication that has been encountered primarily in CKD patients treated with subcutaneous rhEPO and is mediated by neutralizing anti-EPO antibodies that cross-react with endogenous EPO. The peak incidence in 2001 was associated with a change in the formulation of a specific epoetin alfa product (Eprex) containing a new stabilizing agent thought to induce increased immunogenicity of the drug with subcutaneous administration. There have only been rare cases of red cell aplasia after the formulation problem was addressed and Eprex has been administered by an intravenous route.

Loss of rhEPO response during therapy associated with a hemoglobin decline of >0.5 to 1.0 g/dL/week and low reticulocyte count (<10×10⁹/L) leads to clinical suspicion of red cell aplasia. Bone marrow examination reveals absent or severely reduced erythroid precursor cells. Serum EPO antibody testing is required to confirm diagnosis. Discontinuation of drug is indicated. Hematologic recovery occurs in the majority of patients treated with immunosuppressive therapy, such as corticosteroids, daily oral cyclophosphamide, calcineurin inhibitors, or rituximab. Pergesatide, a novel EPOR agonist that does not cross-react with EPO antibodies, has been used successfully in the treatment of some patients. However, this was removed from the US market in 2013 because of increased deaths and cardiovascular events (see below).

Blood doping in sports

There is an extensive literature about athletes using recombinant EPO to improve performance in sports. In the 1980s, some athletes began to transfuse their own blood back into themselves prior to events. Once this was found to help athletic performance, alternative strategies to increase the hemoglobin were sought. When recombinant EPO became available, many capitalized on its availability to raise hemoglobin and increase VO₂max. Some participants in endurance sports (such as cycling, rowing, long-distance running, cross-country skiing, and triathlon) started using EPO. By increasing the hematocrit, it was thought, improvement in oxygen delivery to the muscles would improve endurance. Rules governing the use of EPO in this setting were promulgated, and athletes would

try to circumvent these rules by adopting the use of EPO agents, which could not be detected by laboratories at that time. Great controversy clouded sports such as cycling, and legendary athletes have had their reputations tarnished by discovery of their doping.

In 2017, a provocative update to the blood doping story occurred. Heuberger and colleagues performed the first randomized double-blind trial in which erythropoietin or matched placebo was administered to well-trained cyclists. The study was small and included just 48 participants: 24 to EPO and 24 to placebo. EPO increased the mean hemoglobin concentration from 9.0 to 9.6 mmol/L. EPO increased the maximal power output and VO₂ max, though submaximal parameters, including the mean power output and mean VO₂ consumption, were unchanged. Finally, race times during a day of climbing were no different between groups. The authors conclude that “the more clinically relevant submaximal exercise test performance and road race performance were not affected. This study shows that clinical studies with doping substances can be done adequately and safely and are relevant in determining effects of alleged performance-enhancing drugs.”

rhEPO biosimilars and other erythropoiesis-stimulating agents

The rationale for the development of epoetin biosimilars is cost saving. These products are not fully identical to the original drugs, and documentation of their quality, safety, and efficacy is essential. Immunogenicity and the production of autoantibodies induced by biosimilar epoetins have been associated with pure red cell aplasia. Approved epoetin biosimilars are available for clinical use in Europe.

Pergesatide is a synthetic peptide-based erythropoiesis-stimulating agent (with no sequence similarity to EPO) that stimulates the EPOR dimer and activates similar intracellular pathways that are activated by rhEPO. The dimeric peptide is conjugated to a polyethylene glycol (PEG) moiety, associated with a prolonged half-life of the PEGylated product. Phase 3 clinical trials have been completed for the treatment of anemia in patients with CKD. The FDA initially approved it for use only in CKD patients on dialysis, with a warning and REMS implementation because of increased cardiovascular events compared with rhEPO, which were observed in 2 trials involving predialysis CKD patients. Subsequently, this product was withdrawn in the US in 2013 due to studies showing greater rates of cardiovascular events and death with peginesatide compared with other forms of EPO.

A novel class of erythropoiesis-stimulating agents in clinical development involves HIF stabilization by pharmaco-

logic inhibition of the prolyl hydroxylation of HIF—the transcription factor that controls EPO gene expression—thereby preventing its degradation in the proteasome. An orally bioavailable PHD inhibitor, FG-2216, was reported to increase the plasma EPO level in end-stage renal disease patients (even in anephric hemodialysis patients), suggesting that abnormal oxygen sensing—not a loss of EPO production capacity—plays a role in renal anemia.

Platelet growth factors

Thrombopoietin

TPO is the major HGF that regulates megakaryopoiesis and platelet production. TPO is constitutively synthesized in the liver and kidneys, released into the circulation, and binds to its receptor, MPL (myeloproliferative leukemia virus oncogene), expressed on platelets. Platelet-bound TPO is cleared from plasma, with the remaining TPO available to bind MPL expressed on bone marrow precursors to activate JAK2 tyrosine kinase and downstream intracellular signaling (see video in online edition). The disruption in mice of the gene encoding either TPO or MPL leads to severe thrombocytopenia due to reduced number of megakaryocytes. Serum TPO levels are very high in congenital amegakaryocytic thrombocytopenia (CAMT) due to lack of receptor-mediated uptake. Pecci et al have described mutations in thrombopoietin in patients with CAMT.

Naturally occurring mutations in the gene encoding TPO that lead to increased plasma TPO levels have been found in families with hereditary thrombocytosis. Gain-of-function mutations in the *MPL* gene also have been reported as the basis for congenital or inherited thrombocytosis. Acquired, somatic mutations *MPL* W515L/K have been found in 5% to 10% of patients with essential thrombocytosis and primary myelofibrosis. Homozygous or compound heterozygous inactivating mutations in *MPL* have been reported in association with decreased TPO response in congenital amegakaryocytic thrombocytopenia.

TPO receptor agonists

The development of therapeutic agents to stimulate thrombopoiesis has been of great interest to treat severe thrombocytopenia and bleeding associated with common hematologic conditions, such as chemotherapy-induced thrombocytopenia, MDS, and immune thrombocytopenia (ITP). First-generation recombinant TPOs were investigated in clinical trials involving healthy individuals and patients with chemotherapy-induced thrombocytopenia. The emergence of antibodies that cross-reacted with endogenous TPO prevented the further development of these agents.

Second-generation agents termed TPO receptor agonists (or TPO mimetics), romiplostim and eltrombopag, subsequently were developed and studied in randomized clinical trials in both splenectomized and nonsplenectomized adults with ITP. The efficacy of these agents in increasing platelet counts, achieving durable responses as long as therapy is continued, and reducing the need for other treatments, led to FDA approval of both agents in 2008. The approval indications in Europe by the European Medicines Agency were more restrictive, indicated for splenectomized patients who are refractory to other treatments and considered as second-line treatment for adult nonsplenectomized patients where surgery is contraindicated.

It currently is recommended that TPO receptor agonists be considered only in patients with ITP whose degree of thrombocytopenia and clinical condition increase the risk for bleeding. Long-term continuous therapy is required in the great majority of patients to maintain the platelet response.

Romiplostim

Romiplostim is an injectable peptibody (antibody heavy chain linked to a therapeutic peptide) that consists of a human immunoglobulin G1 Fc domain, linked to a dimer of a 14-amino-acid peptide with no sequence homology to TPO, which binds to and stimulates *MPL* and downstream signaling. In 2 parallel randomized placebo-controlled trials involving splenectomized and nonsplenectomized patients with ITP, a durable platelet response during the 24-week study period was achieved in 38% of romiplostim-treated patients compared with 0% of placebo among splenectomized patients, and 60% of romiplostim-treated patients compared with 4% placebo among non-splenectomized patients. In a subsequent randomized open-label trial involving adults with ITP who had not undergone splenectomy, the rate of platelet response ($>50 \times 10^9/L$) during the 52-week study period was 2.3 times that in the standard-of-care group. Romiplostim-treated patients had a lower incidence of treatment failure and splenectomy, less bleeding, fewer platelet transfusions, and a higher quality of life.

The recommended initial dose of romiplostim is 1 $\mu\text{g}/\text{kg}$ as a weekly subcutaneous injection with dose adjustments weekly by increments of 1 $\mu\text{g}/\text{kg}$ until the patient achieves a stable platelet count of $\geq 50 \times 10^9/\text{L}$. The maximum weekly dose is 10 $\mu\text{g}/\text{kg}$. Treatment goal is to achieve and maintain a platelet count $\geq 50 \times 10^9/\text{L}$ as necessary to reduce the risk for bleeding by using the lowest dose of romiplostim. The development of romiplostim-binding antibodies is rare, and these antibodies are not cross-reactive with TPO.

Eltrombopag

Eltrombopag is an orally bioavailable, nonpeptide, small-molecule TPO receptor agonist that raises platelet counts in a dose-dependent manner. It activates MPL and downstream signaling via JAK2 by association with specific amino acids in the juxtamembrane and transmembrane regions of the receptor. In a randomized double-blind, placebo-controlled trial, once-daily eltrombopag (50 mg) was well tolerated and effective in improving thrombocytopenia. Platelet counts of $\geq 50 \times 10^9/L$ at 6 weeks were achieved in 59% of eltrombopag-treated patients compared with 16% of placebo-treated patients. Eltrombopag-treated patients experienced significantly less bleeding.

The recommended initial dose for most adult patients is 50 mg daily given orally on an empty stomach. Patients with moderate to severe hepatic impairment (Child-Pugh score >7) and individuals of East Asian ethnicity (higher plasma concentrations than white individuals) should be started on a lower dose of 25 mg daily. Response-guided dosing involves biweekly dose adjustment to titrate the eltrombopag dose toward the target platelet level of $\geq 50 \times 10^9/L$. The daily dose should not exceed 75 mg. Antacids, dairy products, and mineral supplements (polyvalent cations such as calcium, iron, aluminum, magnesium) should not be taken within 4 hours of drug ingestion because of reduced absorption.

Monitoring and adverse effects in ITP patients

Romiplostim and eltrombopag should not be used in an attempt to normalize platelet counts. Platelet counts should be measured weekly until stable at $\geq 50 \times 10^9/L$ for at least 4 weeks without dose adjustment, and then monthly thereafter. Dose reduction is recommended when platelets are $>200 \times 10^9/L$. Rebound thrombocytopenia after drug discontinuation, characterized by a transient worsening of thrombocytopenia $10 \times 10^9/L$ below the pretreatment baseline, may occur in 8% to 10% of patients, and may be associated with increased risk of bleeding. If treatment is held or discontinued, it is advisable to monitor platelet counts twice a week for at least 2 weeks and reinitiate other treatments as indicated. Platelet counts usually recover to baseline after several weeks.

The potential adverse effects of these agents include headache, nausea, vomiting, diarrhea, fatigue, nasopharyngitis, and arthralgia. Eltrombopag may be associated with hepatic injury and elevated alanine aminotransferase levels, observed in 10% of patients compared with 7% to 8% of placebo in clinical trials. Serum liver enzymes should be checked before initiation of eltrombopag therapy, every 2 weeks during the dose titration period, and then monthly after establishment of stable dose.

Arterial or venous thromboembolic events were infrequent in long-term studies of romiplostim and eltrombopag in ITP, with an incidence ranging from 2% to 6% and no clear increase in placebo-controlled clinical trials. These events do not appear to correlate with platelet count and tend to occur in patients with other risk factors for thrombosis. A recent study reported the absence of in vivo platelet activation associated with eltrombopag in ITP patients.

Acute renal failure associated with eltrombopag therapy was reported in 2 patients with ITP and antiphospholipid antibodies. Kidney biopsy showed acute thrombotic microangiopathy and tubular injury in 1 patient. Caution is required when considering TPO receptor agonist therapy in patients with ITP and antiphospholipid antibodies.

The true incidence of increased bone marrow reticulin deposition and fibrosis is not known but appears to be infrequent and reversible following discontinuation of therapy. Retrospective analysis of a small number of bone marrow biopsies taken from romiplostim-treated patients in clinical trials and a prospective trial involving pre- and on-therapy bone marrow biopsies showed reticulin increases in several patients, without associated cytopenias. This usually occurred in patients receiving higher doses of romiplostim. In a report of the extended eltrombopag study, 156 bone marrow biopsies were analyzed from 301 patients treated up to 4.5 years. Four specimens (2.6%) exhibited increased reticulin grade MF ≥ 2 . No cytopenias were reported. While on long-term therapy, periodic monitoring for the development of anemia and leukoerythroblastic changes in peripheral blood is advisable.

TPO receptor agonists in aplastic anemia

In 2012, a study reported the results of a phase 2 study involving patients with aplastic anemia refractory to immunosuppression, treated with eltrombopag. Starting dose was 50 mg per day and could be titrated up to 150 mg/day for 12 weeks. Eleven of 25 (44%) had a hematologic response in at least 1 lineage by 12 weeks. Six patients had improved hemoglobin levels, and 3 of them were previously red cell transfusion-dependent. Nine patients had improvement in neutrophil numbers. In an extension phase of this study, 8 patients achieved a multilineage response. Serial bone marrow studies showed that in 8 of the total population in this study new cytogenetic abnormalities developed, including in 5 patients who had changes to chromosome 7. It is now recommended to discontinue eltrombopag for aplastic anemia if this occurs. In 2014, the FDA approved this new indication (refractory aplastic anemia) for eltrombopag. There are ongoing studies of the

addition of eltrombopag to immunosuppressive therapy for newly diagnosed patients with aplastic anemia.

Investigational uses of TPO receptor agonists

Romiplostim and eltrombopag currently are not approved for the treatment of thrombocytopenia because of MDS or any cause of thrombocytopenia other than chronic ITP. Other potential indications are considered investigational at present. The published experience to date in chemotherapy-induced thrombocytopenia is limited. In 12 patients with *MYH9* mutation-related inherited thrombocytopenia, platelet counts improved in 11 patients in response to eltrombopag treatment. The results of several larger clinical trials involving patients with chronic liver disease and MDS have been reported. It appears that in patients with liver disease, hypersplenism, and thrombocytopenia, treatment with eltrombopag to raise the platelet count can lead to more rapid hepatic decompensation (see below).

Thrombocytopenia in chronic liver disease

Eltrombopag was investigated in a randomized placebo-controlled trial for the treatment of thrombocytopenia associated with hepatitis C-related cirrhosis to facilitate antiviral therapy by improving platelet counts. Eltrombopag therapy increased platelet counts allowing for the initiation of antiviral therapy and was well tolerated during the 20-week treatment period.

A more recent placebo-controlled randomized trial involved patients with thrombocytopenia resulting from chronic liver disease, treated for 14 days with eltrombopag before an invasive elective procedure. This trial was terminated because of the occurrence of portal vein thrombosis in 6 patients in the eltrombopag group, compared with 1 patient in the placebo group. Five of the 6 patients treated with eltrombopag had platelet counts $>200 \times 10^9/L$. An association between an increased risk of thrombotic events and platelet counts $\geq 200 \times 10^9/L$ was identified in a post hoc analysis.

In an open-label study of eltrombopag involving 715 patients with thrombocytopenia complicating cirrhosis due to hepatitis C virus infection, 97% of patients were reported to respond with platelets $\geq 90 \times 10^9/L$. No thrombotic complications have been reported to date. Studies investigating the efficacy and safety of eltrombopag for thrombocytopenia associated with chronic liver disease are ongoing.

Avatrombopag (Doptelet) is a new oral TPO receptor agonist approved by the FDA in May 2018 for thrombocytopenia in adults with chronic liver disease who require a rise in platelet count in order to undergo a procedure.

There appears to be less risk of portal vein thrombosis or hepatic decompensation with avatrombopag than with other TPO receptor agonists.

Myelodysplastic syndromes

A phase 1/2 trial involved 44 patients with lower risk MDS and platelets $\leq 50 \times 10^9/L$, treated with single agent weekly romiplostim. A durable platelet response was achieved by 46% of the patients. Increased bone marrow blasts were observed in 9% and AML progression occurred in 2 patients.

The initial results of a randomized, double-blind, placebo-controlled clinical trial involving 250 patients with IPSS low/int-1 risk MDS were reported. Patients were randomized 2:1 to romiplostim 750 µg/week or placebo for a median 21 weeks. Bone marrow biopsies were analyzed after a 4-week washout period. Romiplostim therapy was associated with increased platelet response, reduced bleeding events, and fewer platelet transfusions compared with placebo. Reversible increase in marrow blasts $>10\%$ was observed in 15% of romiplostim-treated patients compared with 3.6% of placebo group patients. The AML rate through 58 weeks of study was 6% for romiplostim, compared with 2.4% for placebo (hazard ratio: 2.51, 95% CI: 0.55–11.47). It is now advised not to use romiplostim in this setting because of this concern over leukemia progression.

Acknowledgments

Much of the text in this chapter is similar to the previous 2 editions' description of hematopoietic growth factors, and we are indebted to those authors (Lyman and Arcasoy, ASH-SAP 5th ed., 2013, and Gerdz and Lichtin, 6th ed., 2016).

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Iron physiology, iron overload, and the porphyrias

HEATHER A. LEITCH AND ELIZABETA NEMETH

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The online version of this chapter contains educational multimedia components on the hormone erythroferrone and on the pathogenesis of porphyria.

Introduction

Iron is vital for survival, but an excess can be harmful, so iron balance must be tightly regulated. Essential functions of iron include oxygen transport and exchange; production of ATP; production of oxygen radicals as well as protection from oxidative damage; DNA synthesis and repair; cellular oxygen sensing; regulation of gene expression; amino acid and lipid metabolism, and many others. The ability of iron to accept and donate electrons allows it to shuttle between the ferrous (Fe^{2+}) and ferric (Fe^{3+}) oxidation states and is essential for its participation in a number of enzymatic reactions. Under physiologic states, iron is mostly bound to proteins and chaperones, but in conditions of iron overload, excess iron catalyzes the formation of free radical ions that may be harmful to cells. Causes of iron overload include repeated blood transfusions, the ineffective erythropoiesis of certain chronic anemias, and mutations in iron-regulatory genes that result in increased iron absorption. This chapter focuses on iron physiology in the normal host and in iron overload states, including hemochromatosis and transfusional iron overload in acquired anemias. Also discussed are the porphyrias as disorders of heme synthesis. Iron deficiency anemia is discussed with the underproduction anemias in Chapter 6.

Regulation of iron homeostasis

Body iron economy

Under normal conditions, dietary iron intake is usually 15 to 25 mg daily, of which only 5% to 10% (1 to 2 mg) is absorbed through the gastrointestinal (GI) tract. A similar amount of iron is lost daily by desquamation of GI epithelial cells (Figure 5-1). The average total body content of iron is 3 to 4 grams, and may be lower in menstruating women. Approximately two thirds of this iron is present in hemoglobin. Iron is stored in cells, predominantly macrophages of the spleen, bone marrow, and liver, but also in hepatocytes, as ferritin or hemosiderin (partially denatured ferritin). At steady state, the serum ferritin level is a reasonably good reflection of total body iron stores. Total storage iron is approximately 1 g in men and 0.5 g in women. Additional iron is found as myoglobin in muscle and in cytochromes and other enzymes (~0.3 g).

Conflict-of-interest disclosure:

Dr. Leitch has received honoraria from, has received research funding from, and/or has served on advisory boards for AbbVie, Alexion, ApoPharma, Celgene, and Novartis. She is a member of the Exjade Speaker's Bureau. Dr. Nemeth is a stockholder of Intrinsic LifeSciences and Silarus Therapeutics and a consultant for La Jolla Pharmaceutical Company, Protagonist Therapeutics, and Keryx Biopharmaceuticals.

Off-label drug use: Off-label use of iron chelation therapy is discussed.

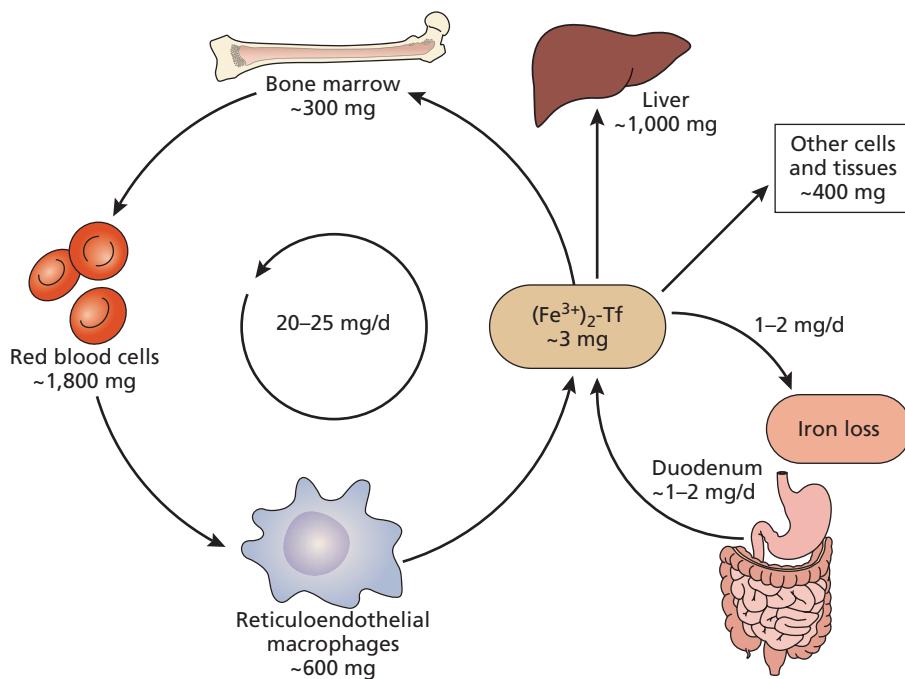


Figure 5-1 Body iron homeostasis. Plasma iron levels are maintained in a relatively narrow range (10 to 30 μM). Iron circulates in plasma bound to transferrin (Tf), which maintains iron in a soluble form, serves as a major route of entry for iron into cells (via the transferrin receptor TfR1), and limits the generation of toxic radicals. The homeostatic system responds to signals from pathways that consume iron (eg, erythropoiesis) and sends signals to the cells that supply iron to the blood stream. Iron is released into the circulation from duodenal enterocytes, which absorb 1 to 2 mg of dietary iron per day, and from macrophages, which internally recycle 20 to 25 mg of iron per day from senescent erythrocytes. While the body regulates processes of iron absorption, storage, and recycling, there is no process for excreting excess iron. Redrawn from Hentze MW et al. *Cell*. 2004;117:285–297, with permission.

Iron is released into the circulation through the iron transporter ferroportin, expressed on the basolateral surface of iron-absorbing enterocytes, on iron-recycling macrophages and on hepatocytes. Ferroportin activity and levels are controlled by the hormone hepcidin: hepcidin binding occludes ferroportin and triggers its degradation, decreasing iron transport into plasma. Hepcidin production itself is regulated by iron: when circulating iron is low, hepcidin levels are low, allowing GI iron absorption to increase and iron stores to be mobilized. When iron is plentiful, hepcidin levels increase and block iron absorption and release from stores.

In the circulation, iron is transported bound to transferrin, and is taken up into cells via the transferrin receptor, with developing red blood cells in the bone marrow utilizing most of the circulating iron. The iron-transferrin compartment is very small ($\sim 3 \text{ mg}$), but it has a high turnover rate so that it transports $\sim 25 \text{ mg}$ of iron daily. Under normal conditions, only around one third of plasma transferrin is iron saturated (reference ranges vary

based on the clinical laboratory but are generally around 20% to 50%).

Iron balance is regulated such that the amount absorbed equals the amount lost. Importantly, there is no physiologically regulated pathway for excretion of excess iron in iron overload. Over the past 15 years, considerable progress has been made concerning the molecular mechanisms underlying the absorption, transport, utilization, and storage of iron. The key proteins discussed are listed in Table 5-1.

Intestinal iron absorption

Iron is found in food as inorganic iron or as heme (iron complexed to protoporphyrin IX). The typical diet consists of 90% inorganic and 10% heme iron, though diets in the industrial world can contain up to 50% heme iron from iron-rich meats. The bioavailability of inorganic but not heme iron is influenced by multiple factors such as other dietary constituents; for example, ascorbic acid (enhances bioavailability), and phytates and polyphenols in cereals and plants (inhibit bioavailability). The rate of iron absorption

Table 5-1 Major proteins involved in iron homeostasis

Protein	Location	Function	Comments
Duodenal cytochrome b (Dcytb)	Duodenal enterocytes, apical surface	Absorption of nonheme iron	Reduces dietary Fe ³⁺ to Fe ²⁺ which is then transported by DMT1
Divalent metal transporter 1 (DMT1)	Duodenal enterocytes, apical surface	Absorption of nonheme iron	Transports Fe ²⁺ across the luminal cell surface
Sodium–hydrogen antiporter 3 (NHE3)	Duodenal enterocytes, apical surface	Absorption of nonheme iron	Generates the H(+) gradient that drives DMT1-mediated iron uptake
Ferroportin (FPN1, SLC40A1)	Ubiquitous expression, but particularly high on: duodenal basolateral surface; hepatocyte cell surface; macrophage cell surface	Iron transport into plasma	Exports iron out of enterocytes, macrophages, and hepatocytes into the plasma
Hephaestin (HEPH)	Duodenal enterocytes, basolateral membrane	Iron absorption	Ferroxidase; oxidizes Fe ²⁺ to Fe ³⁺ ; facilitates iron export via ferroportin into the circulation
Ceruloplasmin (CP)	Plasma and macrophages, liver, central nervous system	Mobilization of stored iron	Ferroxidase; enhances the export activity of ferroportin and loading of iron onto transferrin
Transferrin (Tf)	Plasma	Iron transport in the circulation	Apotransferrin, no bound iron; holotransferrin, 2Fe ³⁺ bound
Transferrin receptor (TfR1)	Cell surface of most cells	Cellular iron uptake	Particularly high expression on erythroid precursors
Ferritin	Intracellular and circulating forms	Iron storage (intracellular form)	Function of the circulating form unknown
Iron regulatory proteins (IRP-1 and -2)	Cytoplasm	Regulate production of proteins involved in cellular iron uptake, storage and export	Bind to iron-responsive elements (IRE) on mRNA; stabilize mRNAs with 3' IRE (TfR1, DMT1); decrease translation of mRNAs with 5' IREs (ferritin, ferroportin, HIF-2α, ALAS2)
Hepcidin (HAMP)	Hormone produced mainly by the liver	Regulates plasma iron by controlling iron absorption and release from stores	Occludes ferroportin and causes its degradation
Erythroferrone (ERFE)	Hormone produced by erythroid precursors	Regulates hepcidin in response to erythropoietic stimulation	Suppresses hepcidin, allowing iron absorption and mobilization of stored iron
HFE	Ubiquitous expression, prevalent function in hepatocyte	Regulates hepcidin in response to iron stimulation	A protein mutated in most cases of hereditary hemochromatosis
Hemojuvelin (HJV)	Hepatocyte cell surface	Regulates hepcidin in response to iron stimulation	A BMP coreceptor
Transferrin receptor 2 (Tfr2)	Hepatocyte cell surface; erythroid precursors	Regulates hepcidin in response to iron stimulation; modulates EPOR on erythroid precursors	Holotransferrin sensor
Bone morphogenetic proteins (BMPs)	Growth factors	Regulate hepcidin baseline and response to iron stimulation	Produced by the liver sinusoidal endothelial cells
BMP receptors (ALK2, ALK3; ACTRIIA and BMPR2)	Hepatocyte cell surface	Regulate hepcidin baseline and response to iron stimulation	Activate SMAD 1/5/8 pathway to increase hepcidin transcription
Sons of mothers against decapentaplegic (SMAD) proteins	Intracellular signal transduction and transcription factors	Regulate hepcidin baseline and response to iron stimulation	Phospho-SMAD 1/5/8 complexing with SMAD 4 promotes hepcidin gene transcription
Transmembrane protease serine 6 (TMPRSS6)	Hepatocyte cell membrane	Regulates hepcidin response to iron deficiency	Serine protease; decreases BMP signaling by cleaving HJV
IL-6, IL-6 receptor	Cytokine and its receptor	Regulate hepcidin in response to inflammation	Increase hepcidin transcription by activating the JAK/STAT pathway
Hypoxia-inducible factor 2α (HIF2α)	Intracellular transcription factors	Regulates iron absorption	Activates duodenal transcription of ferroportin, DMT1 and Dcytb; may contribute to iron overload in ineffective erythropoiesis; regulates erythropoietin production in the kidneys

is influenced by several factors, including body iron stores, the degree of erythropoietic activity, and the presence of inflammation. Iron absorption increases when stores are low or when erythropoietic activity increases, such as during anemia or hypoxemia. Conversely, the physiologically appropriate response to iron overload is downregulation of intestinal iron absorption; this downregulation fails in patients with hereditary hemochromatosis or chronic iron-loading anemias.

Iron is absorbed in the intestine via 2 pathways: one for inorganic iron and the other for heme-bound iron. Little is known about heme iron absorption. Nonheme iron in the diet is largely in the form of ferric oxyhydroxides (Fe^{3+}), but the intestinal epithelial cell apical iron importer, divalent metal transporter 1 (DMT1 or SLC11A2), transports only ferrous iron (Fe^{2+}). Iron must therefore be reduced to be absorbed, and this is facilitated by a ferrireductase duodenal cytochrome B (Dcytb). Once transported across the apical border of the enterocyte, iron may be stored within the cell. For this purpose, iron is oxidized to Fe^{3+} by the H-subunit of ferritin and stored in this form. Eventually, the cell senesces and sloughs off into the feces, and stored iron is lost to the system. Alternatively, iron may be transported across the basolateral membrane into the portal circulation via ferroportin. Ferroportin 1 (FPN1) is the only known iron exporter in mammals and, like DMT1, transports only ferrous iron. Once reduced, ferrous iron is transported across the basolateral membrane by ferroportin, then oxidized to ferric iron by hephaestin. Intestinal iron absorption is regulated by hepcidin, as discussed above. During iron deficiency and anemia, at least in animal models, intestinal iron absorption is further increased through the activity of the intestinal HIF2 α . HIF2 α promotes transcription of ferroportin, DMT1 and Dcytb, leading to increased apical and basolateral transport of iron. Activation of this pathway may also contribute to the development of iron overload in anemias with ineffective erythropoiesis.

Cellular iron uptake, storage, and recycling

Each molecule of transferrin binds 2 ferric (Fe^{3+}) iron atoms. Diferric transferrin (holotransferrin) binds to the transferrin receptor (TfR1) on target cells and enters by receptor-mediated endocytosis; iron is then released from the Tf-TfR1 complex by acidification and transported into the cytoplasm by DMT1. Apo-Tf and TfR1 are recycled to the cell surface. Regulation of the synthesis of multiple proteins involved in iron physiology, including TfR1, DMT1, FPN1, and ferritin, is controlled at a posttranscriptional level by influencing mRNA stability or translation. The mRNAs of these proteins contain iron response

elements (IREs), conserved nucleotide sequences with a stem-loop structure that binds iron regulatory protein 1 (IRP-1) and IRP-2. The mRNAs for ferritin and FPN1 have IREs in the 5' untranslated region (UTR), and the mRNA for the TfR1 and DMT1 have IREs in the 3' UTR. When a cell is iron-deficient, IRPs bind to IREs. Binding to the 3' IREs stabilizes the mRNA (TfR1 or DMT1) and allows increased cellular iron uptake. Binding of IRPs to the 5' UTR of ferritin or FPN mRNA, decreases translation of these mRNAs, resulting in less storage and export of iron in an iron-deficient cell. When intracellular iron concentrations increase, the fate of the 2 IRPs differs: IRP-1 is converted from an RNA-binding protein into an aconitase, whereas IRP-2 is degraded by a ubiquitin ligase complex. As a result, IREs are not occupied by IRPs, leading to decreased production of the iron uptake proteins TfR1 and DMT1 and increased translation of ferritin and ferroportin, protecting the cell from iron excess.

Within each cell, iron is destined for mitochondrial heme synthesis, iron-sulfur cluster synthesis, incorporation into iron-containing enzymes, or is stored within ferritin. Erythroid cells are by far the most avid consumers of iron, and utilize it to synthesize heme, which complexes with globin proteins, forming hemoglobin. In erythroid cells, the first step in heme synthesis, the condensation of glycine and succinyl coenzyme A is catalyzed by aminolevulinic acid synthase 2 (ALAS2), an enzyme whose production is regulated by iron availability via the IRE-IRP system. ALAS2 mRNA contains a 5' IRE, thus its translation is increased when cellular iron increases, providing a link between iron availability and heme synthesis. Erythrocytes survive in the circulation for approximately 120 days, after which aging red blood cells are phagocytized by macrophages of the spleen and liver. Hemoglobin is catabolized in macrophages, releasing heme. Heme is then degraded by the enzyme heme oxygenase to produce iron, biliverdin, and carbon monoxide. Iron is either stored within ferritin or released into the circulation via ferroportin.

The main form of cellular iron storage is ferritin, a 24-subunit nanocage that binds iron and renders it insoluble and redox inactive. Iron is recovered from ferritin through the process of ferritinophagy, mediated by the autophagy receptor NCOA4. Under conditions of high intracellular iron, NCOA4 is degraded through the action of HERC2 ubiquitin E3 ligase, and ferritin remains stable. When the cell is iron-deficient, NCOA4 accumulates and triggers autophagy of ferritin, eventually resulting in the release of iron from lysosomes. Interestingly, the process of ferritinophagy is also utilized by erythroid precursors to deliver iron for hemoglobin synthesis. In contrast to intracellular ferritin, serum ferritin has a different composition

of subunits, is relatively iron-poor, and its function is not understood.

Regulation of systemic iron physiology

Hepcidin is a 25-amino-acid peptide hormone produced mainly by the liver and is the major regulator of iron absorption and storage. Hepcidin regulates cellular iron egress, causing occlusion of ferroportin, and its internalization and degradation. In this way, elevated levels of hepcidin inhibit iron absorption from the GI tract and prevent the release of iron from hepatocytes and macrophages (Figure 5-2). Hepcidin production is strongly regulated by iron (both circulating and stored), erythropoietic activity and inflammation. Most of the mechanistic understanding of hepcidin regulation has been derived from animal models.

Hepcidin transcription is increased proportional to iron loading, which prevents further iron absorption and ensures the maintenance of body iron balance. Conversely, hepcidin levels are decreased in iron deficiency to allow greater iron absorption and correction of the body iron deficit. Iron-dependent hepcidin regulation is mediated by the bone morphogenetic protein (BMP) pathway (Figure 5-3). BMPs are members of the TGF- β superfamily and have pleiotropic roles in the body. BMP ligands bind to com-

plexes of type I and type II serine and threonine kinase receptors, which phosphorylate receptor-activated SMADs 1/5/8. These associate with SMAD 4, forming an activated SMAD transcription factor complex which increases hepcidin transcription. Another key hepcidin-regulatory molecule is hemojuvelin (HJV), which functions as a BMP co-receptor and facilitates interaction of specific BMP ligands and receptors. Mutations in HJV result in severe hepcidin deficiency and juvenile (type 2) hemochromatosis.

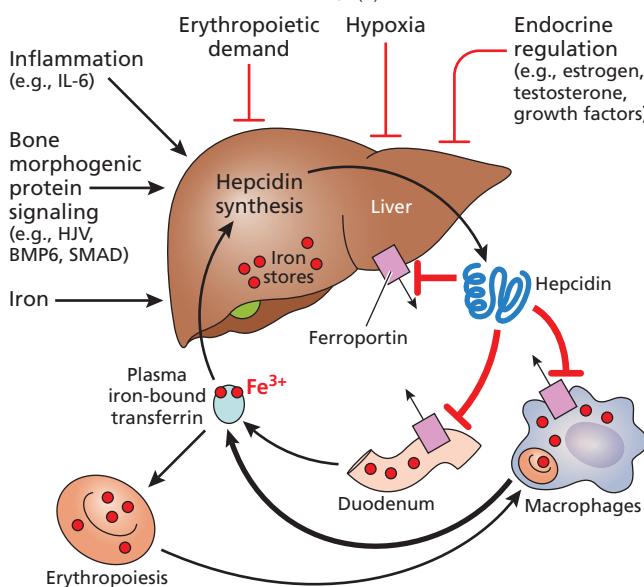
Several BMP ligands were reported to induce hepcidin expression in vitro, but mouse models have shown that BMP6 and BMP2 are important in vivo. Specifically, BMP6 and 2 are produced by the sinusoidal endothelial cells in the liver, and act on hepatocytes to maintain baseline hepcidin expression. Furthermore, BMP6 production is induced by iron loading, mediating hepcidin induction in response to increased stores.

Extracellular iron-sensing (holo-transferrin sensing) is dependent on TFR1, TFR2, and HFE, all expressed on hepatocytes. HFE is an MHC class I-like protein, identified as a gene mutated in the most common form of hereditary hemochromatosis. HFE interacts with TFR1, but is displaced from the complex by holo-Tf binding to TFR1. Instead, HFE interacts with ALK3, a BMP receptor type I, and prevents its ubiquitination and degradation, thus stabilizing ALK3 protein on the surface of hepatocytes proportional to the concentration of holo-Tf. When holo-Tf concentrations are high, TFR2 protein is also stabilized by binding holo-Tf, and likely interacts with HFE and HJV. Thus, it is thought that increasing holo-Tf concentration leads to the formation of a multiprotein complex centered on the BMP pathway and potentiates SMAD signaling (Figure 5-3). In hereditary hemochromatosis, defects in hepatocyte iron sensing lead to inappropriately low levels of hepcidin for the degree of iron present.

In iron deficiency, BMP signaling and hepcidin production is downregulated by the hepatocyte cell surface serine protease TMPRSS6 (transmembrane protease serine S6). TMPRSS6 is stabilized during iron deficiency and cleaves HJV, leading to decreased SMAD signaling. Mutations in TMPRSS6 lead to inappropriately elevated hepcidin concentration, and result in iron-refractory iron deficiency anemia.

Hepcidin is potently increased by inflammation, and this is mediated by interleukin (IL)-6 signaling (the JAK/STAT pathway), with synergistic contribution from the BMP pathway. Increased hepcidin causes hypoferremia, a host defense mechanism against extracellular pathogens, particularly gram-negative bacteria, whose rate of growth is strongly influenced by iron. Chronically elevated hepcidin and consequent hypoferremia result in development

Figure 5-2 Regulators of iron balance. The hormone hepcidin regulates plasma iron concentration by controlling ferroportin levels on iron-exporting cells including duodenal enterocytes, recycling macrophages of the spleen and liver, and hepatocytes. Hepcidin production is regulated by multiple stimuli: intracellular and extracellular iron concentration increase hepcidin transcription, as does inflammation, whereas erythropoietic activity suppresses hepcidin production. With permission from Steinbicker AU, Muckenthaler MU. *Nutrients*. 2013;5(8):3034–3061.



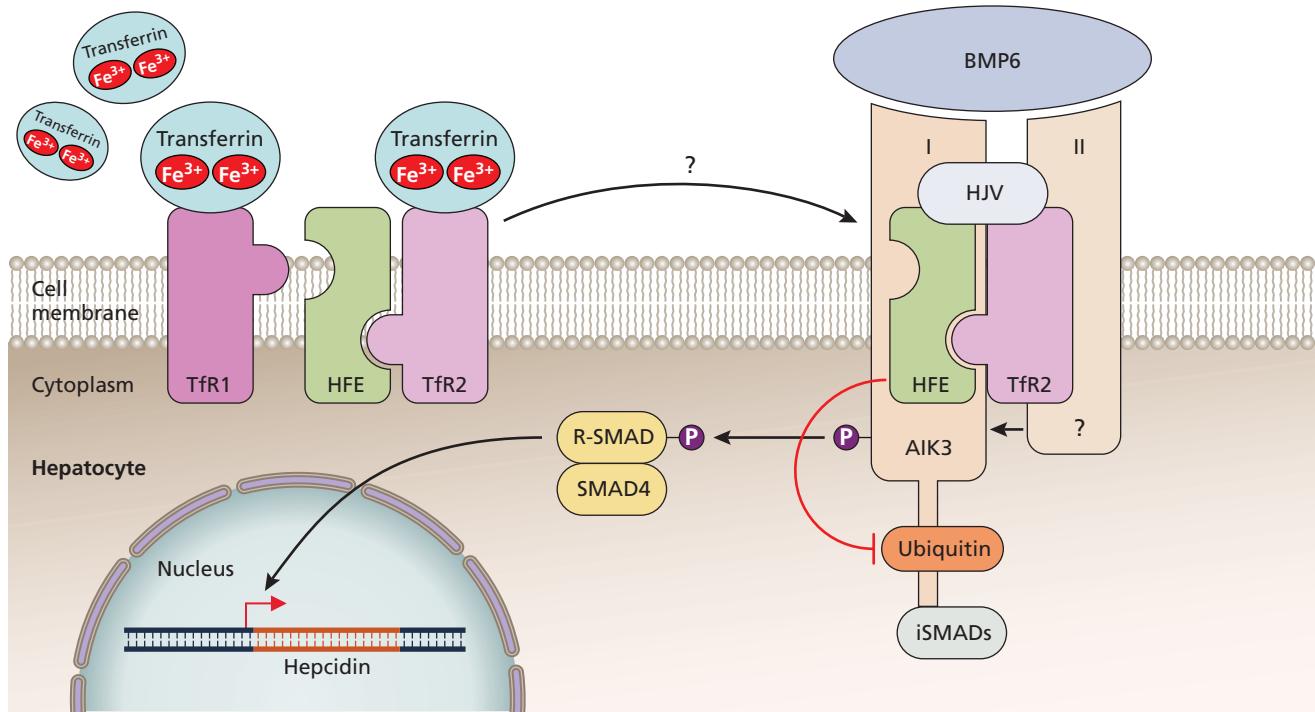


Figure 5-3 A model of the regulation of hepatic hepcidin expression. Regulated protein-protein interactions among HFE, TfR2, HJV (proteins mutated in HH), BMP receptors, and BMP ligands play a critical role in the “sensing” of transferrin-bound iron (Fe) to control hepcidin expression in hepatocytes. HFE binds to BMP receptor type I (Alk3) to prevent its ubiquitination and proteasomal degradation. As a result, expression of ALK3 is increased on the cell surface, activating BMP/SMAD signaling and hepcidin transcription. BMP ligands that regulate hepcidin production are secreted by the liver sinusoidal endothelial cells, and the rate of BMP6 production is regulated by liver iron stores. Redrawn from Muckenthaler MU. *Blood*. 2014;124:1212–1213, with permission.

of anemia of inflammation (anemia of chronic disease). Multiple hepcidin agonists and antagonists are under clinical development for the treatment of disorders of inappropriately low or high hepcidin levels, respectively.

Hepcidin production is suppressed by an increase in erythropoietic activity, for example after hemorrhage or administration of erythropoietin. Erythropoietic hepcidin suppression is mediated by the recently described hormone erythroferrone (ERFE, see video file in online edition). ERFE is produced by erythroblasts in response to EPO during stress erythropoiesis. ERFE acts as a BMP6 trap, leading to decreased hepatic SMAD 1/5 phosphorylation and hepcidin expression, thus allowing iron absorption and release from storage to increase, providing greater iron availability for erythropoiesis.

Hereditary hemochromatosis and other iron overload disorders

Iron deposition in body tissues or organs is referred to as iron overload (hemosiderosis). Iron overload may lead to iron-induced injury in affected body tissues. Hereditary

hemochromatosis is a congenital cause of iron overload resulting from increased gastrointestinal iron absorption. Other etiologies of iron overload are discussed below (Table 5-2).

The toxicity of excess iron is mediated by its ability to catalyze generation of reactive oxygen species (ROS). Once the transferrin saturation is elevated (from 70% to 80%–85%, depending on the study), nontransferrin-bound iron (NTBI) appears in the circulation. A portion of NTBI is redox active and referred to as labile plasma iron (LPI), which promotes formation of ROS. NTBI is taken up by cells expressing NTBI transporters, leading to cellular iron overload. Excess intracellular iron damages subcellular components (Figure 5-4) and eventually causes organ dysfunction.

HFE hemochromatosis

Epidemiology and genetics

HFE hemochromatosis is the most common form of hereditary hemochromatosis. It is prevalent in individuals of Northern European descent because of the pres-

Table 5-2 Causes of iron overload

Condition	Cause	Mechanism	Comments
1. Hereditary conditions			Increased iron absorption leads to elevated Tf saturation and appearance of NTBI; hepatocytes express the highest levels of NTBI transporters; therefore, hepatic iron overload usually predominates
i) Hereditary hemochromatosis		Impairment in the hepcidin/ferroportin axis	
<i>HFE</i> hemochromatosis	Point mutations in the <i>HFE</i> gene	Relative hepcidin deficiency	Amino acid substitutions; found primarily in Caucasians
<i>TFR2</i> hemochromatosis	Mutations in <i>TFR2</i>	Relative hepcidin deficiency	Found in multiple ethnicities
Hemojuvelin (HJV) hemochromatosis	Mutations in HJV or compound heterozygote with <i>HFE</i>	Absolute hepcidin deficiency	Juvenile hemochromatosis
Hepcidin (HAMP) hemochromatosis	Mutation in HAMP	Absolute hepcidin deficiency	Juvenile hemochromatosis
Ferroportin disease			
Classical	Heterozygous missense mutations in ferroportin	Unable to export iron	Loss of function, “macrophage type”
Nonclassical		Resistant to hepcidin	Gain of function, “hepatic type”
ii) Other congenital iron overload syndromes			
African iron overload	Possible polymorphism in ferroportin gene, compounded by high iron consumption	Increases transferrin saturation and ferritin	Hepatic and RES iron overload
Aceruloplasminemia	Mutations in ceruloplasmin gene	Affects ferroxidase activity	Impairs ability to mobilize iron from macrophages and hepatocytes Neurological manifestations, DM, anemia
Atransferrinemia	Mutations in Tf gene	Unable to deliver iron to erythroid precursors	Increased GI iron absorption and deficiency of Tf leads to high NTBI and loading of parenchyma
iii) Congenital anemias (eg, β -thalassemia, hereditary sideroblastic anemia)		Ineffective erythropoiesis \pm transfusions	Increased GI absorption \pm RES overload Discussed in Chapters 6 and 7
2. Acquired clonal conditions (eg, myelodysplastic syndromes, myelofibrosis)		Transfusions \pm ineffective erythropoiesis	RES overload \pm increased GI absorption Discussed in Chapters 18 and 19
3. Iatrogenic	Inappropriate iron supplementation		Intravenous iron repletion for the anemia of renal failure; oral iron supplements for noniron deficiency causes of anemia

DM, diabetes mellitus; *HFE*, homeostatic iron regulator, the gene affected in hereditary *HFE* hemochromatosis; GI, gastrointestinal; NTBI, non-transferrin-bound iron; RES, reticuloendothelial system; Tf, transferrin.

ence of the autosomal-recessive founder allele, C282Y. It is distinctly uncommon in other ethnicities. Significant variation exists between the genotypic and phenotypic expression of *HFE* hemochromatosis because of the presence of genetic modifiers or environmental factors.

A G-to-A mutation at nucleotide 845 of *HFE* leads to a cysteine-to-tyrosine substitution at amino acid 282, the

C282Y mutation. In some geographical areas (eg, the northern United Kingdom and Ireland), 10% to 15% of white persons are heterozygous for this mutation (C282Y/WT), though the clinical expression of iron damage is rare. About 0.5% are homozygous (C282Y/C282Y), but homozygotes account for 60% to 90% of clinical cases of hereditary hemochromatosis. Although biochemical abnormalities such as an elevated transferrin saturation or ferritin

A Cellular consequences of labile iron

- Iron has an ability to transfer electrons
(Fenton reaction: $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH}$)
- Production of free O_2^- radicals:

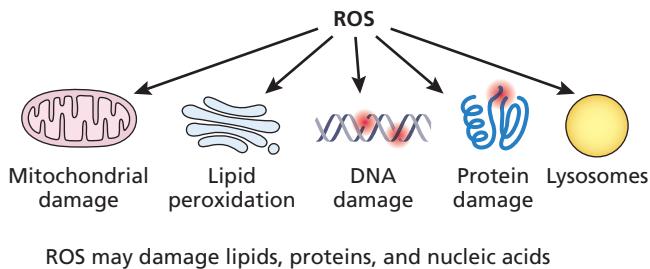
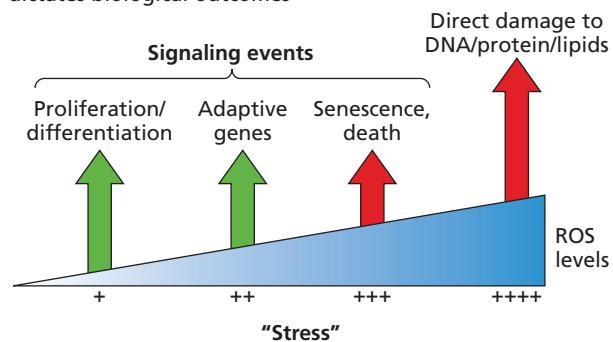
**B** Model: mitochondrial ROS signaling dictates biological outcomes

Figure 5-4 Cellular responses to oxidative stress. Once transferrin saturation is elevated (70% to 85%), nontransferrin-bound iron (NTBI) appears in the circulation and is taken up by NTBI transporters on parenchymal cells. Excess iron in the circulation and intracellularly through Fenton chemistry causes the formation of reactive oxygen species (ROS) that damage cellular and subcellular components (A). Cellular consequences may include cell death, or mutation and malignant progression (B). (A) Modified from Slotki I, Cabantchik ZI. *J Am Soc Nephrol*. 2015;26(11):2612–2619, with permission. (B) Redrawn from Hamanaka RB et al. *Trends Biochem Sci*. 2010;35:505–513, with permission.

level rarely may be present in heterozygotes, few develop clinical features of iron overload in the absence of other risk factors, such as alcoholic hepatitis (Table 5-3).

A second mutation involves a G-to-C substitution at HFE nucleotide 187, leading to a histidine-to-aspartic-acid substitution at amino acid 63 (H63D). Up to 30% of Caucasians in some geographical areas are heterozygous for this allele. H63D is less penetrant than C282Y, and only a small minority of homozygotes (H63D/H63D) develop clinical features of iron overload. Heterozygotes for the H63D mutation (H63D/WT) rarely develop biochemical or clinical evidence of iron overload. Compound heterozygotes (C282Y/H63D) occasionally may develop mild

iron overload and should be evaluated for coexisting risk factors if hemochromatosis is clinically expressed. In the United States, 15% to 30% of patients with clinical hemochromatosis have no identifiable HFE mutation.

Although homozygosity for the C282Y allele accounts for up to 90% of clinical hereditary hemochromatosis, the true phenotypic penetrance of HFE mutations remains a matter of debate. In a population screening study, 50% of C282Y homozygotes developed disease expression, typically by age 60. In a pedigree study of homozygous family members of known affected individuals, 85% of males and 65% of females had biochemical evidence of iron overload. Despite this, only 38% of males and 10% of females had disease-related symptoms, and 15% had fibrosis or cirrhosis on liver biopsy. Other studies suggested the clinical penetrance may be lower; symptoms were no more prevalent in homozygotes than in an unaffected control population, and the penetrance was estimated at less than 1%. The true clinical penetrance is uncertain but probably between 1% and 25%. Much of the variability in estimates is a result of different populations studied (blood donors vs preventive care clinics vs the general population vs family members of affected individuals) and how clinical penetrance was defined (iron studies vs liver function tests vs clinical symptoms vs liver biopsy).

Clinical presentation and diagnosis

The classic finding of a male with skin bronzing, hepatomegaly, and diabetes is an advanced (and now rare) presentation. Patients often present for evaluation of abnormal iron studies identified during routine physicals, as part of screening when affected relatives are identified, or when iron panels are drawn for other reasons. Despite a relatively common finding of abnormal biochemical iron tests, the clinical expression of iron-related organ damage is rare. Nevertheless, early diagnosis is important to prevent iron overload and avoid end-organ complications. The clinical presentation is varied and often nonspecific—such as fatigue, weakness, abdominal pain, arthralgias, and mild elevation of liver enzymes. Endocrine organs are commonly affected, and diabetes, hypothyroidism, and gonadal failure may occur. Both the mechanical and conduction systems of the heart may be affected, resulting in heart failure or arrhythmias. However, the earliest clinical sign of tissue damage is alterations in liver function tests and the earliest histologic sign is hepatic fibrosis. Iron-induced liver damage remains the most recognized complication of untreated disease (Figure 5-5).

The transferrin saturation in patients with hereditary hemochromatosis is higher than in normal individuals but

Table 5-3 Prevalence of *HFE* genotypes among patients with hereditary hemochromatosis

Genotype	Prevalence among patients with hereditary hemochromatosis	Gene frequency in the population	Penetrance
C282Y/C282Y	60%–90%	0.5%*	13.5%†
C282Y/H63D	0%–10%		Low
C282Y/WT	Rare	10–15%*	Low
H63D/H63D	0%–4%		Lower
H63D/WT	Rare	20%‡	Not penetrant
WT/WT	15%–30%		Unknown
Private mutations	Rare		Unknown

Adapted from Cogswell ME et al, *Am J Prev Med*. 1999;2:134–140.

WT, wild type.

C282Y refers to a cysteine to tyrosine substitution at amino acid position 282. H63D refers to a histidine to aspartic acid substitution at amino acid position 63.

*Caucasian population.

†European; clinical iron overload in all but C282Y homozygotes should prompt a search for contributing factors to iron overload.

‡Global population.

shows considerable variability. A transferrin saturation >50% in males or >45% in females should prompt a fasting measurement and measurement of the serum ferritin level. Ferritin, though imperfect, is a reasonable surrogate for total body iron stores. Ferritin can be elevated in other conditions, including metabolic syndrome, inflammatory states, acute or chronic hepatitis, alcoholic liver disease, and others. In a population-based screening program performed through the Centers for Disease Control and Prevention, 11% to 22% of individuals with an elevated serum transferrin saturation had a concurrent elevation in serum ferritin level.

Molecular genotyping of the *HFE* locus, now a readily available test, should be considered if the diagnosis remains in question after secondary causes of iron overload have been ruled out or if affected family members exist.

Liver biopsy is the historical gold standard for diagnosis of hepatic iron overload. Biopsy provides information on iron content and distribution and whether fibrosis or cirrhosis has developed. Liver biopsy has been recommended for C282Y homozygotes with abnormal liver function tests or ferritin >1,000 ng/mL to evaluate for cirrhosis and can also be considered if a strong suspicion of significant iron overload exists, despite a negative evaluation for *HFE* mutations or other primary or secondary causes. If serum ferritin is <1,000 ng/mL, cirrhosis is rare. Iron distribution is primarily within hepatocytes (parenchymal), sparing Kupffer cells. A Perls stain of grade 3 or 4, a liver iron concentration (LIC) of 80 mmol/g (4.5 mg/g) dry weight or greater, or a hepatic iron index score 1.9 or greater (hepatic iron in mmol/g divided by patient age) all confirm the presence of increased body iron stores. Another method of estimat-

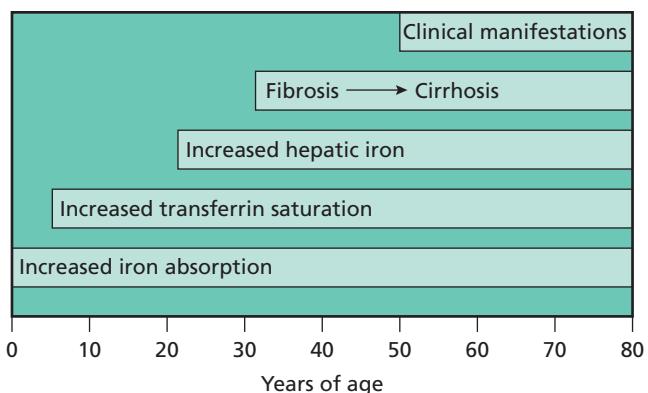


Figure 5-5 The natural history of hemochromatosis in relation to the liver in those individuals who develop clinical manifestations of iron overload. An increase in the percent saturation of transferrin can be detected in children homozygous for hemochromatosis. Increased liver iron stores generally can be detected in homozygous men by the end of the second decade. The serum ferritin concentration increases as hepatic iron stores increase. Hepatic fibrosis can be detected early in the fourth decade. Clinical manifestations generally occur in the fifth decade or later.

ing storage iron is by phlebotomy. If more than 4 g of iron (about 16 units of blood) can be mobilized without the patient becoming iron deficient, iron stores are at least 4 times normal. Liver biopsy is performed less frequently now that confirmatory genotyping has become readily available. Drawbacks of liver biopsy include its invasive nature and inhomogeneous distribution of storage iron, leading to inaccurate estimates of LIC.

Techniques including R2* or T2* magnetic resonance imaging (MRI) or superconducting quantum interference

device (SQUID) susceptometry are noninvasive methods increasingly used for documenting organ iron overload. MRI is available in an increasing number of centers and assesses iron deposition in the liver and heart, and more recently the endocrine organs. SQUID is available in only a few centers worldwide. Newer techniques for measuring iron overload, such as dual energy computed tomography, have also been described. Fibroscan is increasingly used to assess for hepatic fibrosis.

Treatment

Iron depletion prior to the occurrence of end-organ complications such as cirrhosis results in normal life expectancy. Phlebotomy of 1 unit of blood (400 to 500 cm³ of whole blood; 200 to 250 mg of iron) should be initiated at up to weekly intervals and then tapered in frequency to maintain a ferritin level around 50 ng/mL, provided the hematocrit is maintained above 33% to 35%. Normal adults become iron deficient after 4 to 6 phlebotomies because the typical 1 g of iron stores is depleted. Patients with 4 g of storage iron do not become iron deficient until 16 to 20 phlebotomies have been performed. The clinical benefit of aggressive phlebotomy in moderate iron overload is less clear.

Phlebotomy is often effective at improving a patient's overall sense of well-being, resolving fatigue and malaise, normalizing skin pigmentation, and reducing elevated liver enzymes. Arthralgias, diabetes, and hypogonadism may not resolve, and cirrhosis or risk for hepatocellular carcinoma may not be reversed. It is important that patients understand that arthralgias in particular may not improve or may even worsen with phlebotomy.

Phlebotomy usually is not indicated and only infrequently performed during adolescence. If an isolated increase in fasting transferrin saturation is identified during screening, ferritin level should be monitored at 3- to 6-month intervals and phlebotomy initiated when the ferritin is >300 ng/mL in males or >200 ng/mL in nonpregnant females. Avoidance of alcohol and exogenous medicinal iron or iron-containing vitamins should be stressed. Dietary change aimed at avoiding iron-containing foods is often not necessary as long as patients are compliant with phlebotomy. Patients should be warned about the risks of eating raw seafood, undercooked pork, or unpasteurized milk because the incidence of severe *Vibrio vulnificus* and *Yersinia enterocolitica* infections increases in iron overload. The risk for mucormycosis may also increase if they begin chelation therapy. Iron chelation therapy should be considered if phlebotomy is contraindicated. Treatment of hepatic or other complications of iron overload

is essential. Once cirrhosis develops, there is a >200-fold increased risk of hepatocellular carcinoma compared with the general population. Serial ultrasounds with or without measurement of α-fetoprotein may be employed to screen for hepatocellular carcinoma in at-risk individuals. Liver transplantation has been performed for end-stage liver disease in these patients.

Screening

Population screening for hereditary hemochromatosis is controversial and currently not recommended. However, early screening of at-risk individuals or families by measurement of fasting transferrin saturation, ferritin level, and HFE genotyping should be discussed. The possibility of genetic discrimination should be discussed before screening; for this reason, some authorities recommend against genetic screening before adulthood.

Other autosomal-recessive forms of hereditary hemochromatosis

Patients with HFE hemochromatosis rarely present before the fourth decade of life. Clinically significant iron overload in the 20s and 30s is more likely the severe, early onset autosomal-recessive disorder juvenile hemochromatosis, which occurs due to recessive loss-of-function mutations in HJV or hepcidin. Juvenile hemochromatosis characteristically presents with life-threatening heart failure and polyendocrinopathies (eg, hypogonadotropic hypogonadism and impaired glucose tolerance or diabetes mellitus) more frequently than liver dysfunction or other clinical manifestations. Patients often require intensive management of cardiac complications but may recover fully with an aggressive iron depletion regimen. Recessive mutations in TfR2 are rare, and the disease phenotype is indistinguishable from HFE hemochromatosis other than a near-complete penetrance and possible presentation at an earlier age. Like HFE hemochromatosis, a common feature of these disorders is a relative deficiency of hepcidin for the degree of iron overload; the severity of the disease phenotype roughly correlates with the magnitude of hepcidin deficiency.

Neonatal hemochromatosis presents as perinatal liver failure and widespread systemic parenchymal iron deposition, but it is likely not a primary disorder of iron balance and appears to be a consequence of alloimmune hepatitis from a fetal-maternal antigen incompatibility. Treatment with intravenous immunoglobulin beginning in midgestation mitigates the severity of iron overload in newborns of mothers with a prior affected child.

Ferroportin disease

Iron overload resulting from autosomal dominant mutations of FPN1 is known as ferroportin disease. The most frequent form is from mutations that result in partial loss of FPN1 function (“classical ferroportin disease”) either due to an impairment in transport function or mistrafficking and decreased protein stability. Serum ferritin is often increased in the presence of a low-normal transferrin saturation or hemoglobin. These patients typically have substantial Kupffer cell iron storage early in their course. They often sustain an early decrease in serum iron and hemoglobin during phlebotomy, which may limit their tolerance of treatment.

Patients with a gain-of-function mutation (“nonclassical ferroportin disease”) have clinical and histopathological features similar to autosomal-recessive forms of hemochromatosis. Characteristically, mutations affect the ability of hepcidin to bind or induce ubiquitination and degradation of FPN1, leading to a hepcidin-resistant phenotype. The patients display a spectrum of clinical phenotype, and though many require only careful monitoring, some may develop significant hepatic iron overload or other complications such as arthropathy. It may be reasonable to assess tissue iron levels with imaging and institute treatment in affected individuals.

Other causes of iron overload

Many chronic anemias, particularly the thalassemias, are associated with clinically significant iron overload (Table 5–2). Iron overload in these patients can be due to transfusion, increased iron absorption, or both. Ineffective erythropoiesis, the intramedullary death of developing red blood cells, leads to inappropriately increased iron absorption through suppression of hepcidin production, likely via erythrocferrone (see video file in online edition). Ineffective erythropoiesis can lead to significant iron-related morbidity even in the absence of transfusion in patients with thalassemia intermedia and other anemias. Blood transfusions are the predominant cause of iron overload in patients with thalassemia major, aplastic anemia, pure red cell aplasia, myelodysplastic syndromes (MDS), and sickle cell anemia.

Less severe forms of iron overload have been described with alcoholic cirrhosis, hepatitis C virus infection, non-alcoholic steatohepatitis, and porphyria cutanea tarda. In some of these disorders, the frequency of HFE mutations is higher than would be predicted by chance and likely contributes to the risk of iron overload. Hereditary aceruloplasminemia may mimic hemochromatosis but is characterized by normal transferrin saturation and the presence

of neurologic deficits such as ataxia and dementia. Symptoms appear in adulthood, making an early diagnosis difficult. This disorder is extremely rare, and the exact incidence is unknown, but it may be more prevalent in Japan. As ceruloplasmin has ferroxidase activity that is important for the release of iron from macrophages, patients with a mutated gene may accumulate excess iron. Finally, aggressive intravenous iron administration in conditions such as the anemia of renal failure has been reported to result in iron overload.

Iron chelation therapy

The management of secondary iron overload may be challenging. Anemia often exists, requiring red blood cell transfusions and making phlebotomy impractical. In some cases, erythropoiesis stimulating agents such as erythropoietin can be used to increase the hematocrit to a range safe for phlebotomy. Splenectomy may decrease transfusion requirements in some anemias. Treatment of the underlying condition, as in aplastic anemia, MDS, or myelofibrosis should be undertaken if possible.

In situations where offloading excess iron is desirable but phlebotomy cannot be used, iron chelation therapy may be considered. There is considerable experience with this treatment in the hemoglobinopathies, where offloading organ and total body iron has been demonstrated to prevent and even reverse iron overload and organ dysfunction. There is increasing experience with iron chelation therapy in acquired anemias, conditions in which at least some patients appear to benefit from reduction of iron overload. There is a body of preclinical evidence suggesting that some benefit of iron chelation therapy in these conditions may be from removal of labile iron and its toxic effects; labile iron is suppressed very rapidly with chelation, within minutes to hours (Figure 5–4), as opposed to removal of total body iron, which takes months to years.

All chelators have potential side effects and require appropriate monitoring, as per the product monographs, and as summarized in Tables 5–4 and 5–5. The first available iron chelation agent was deferoxamine, which has been used extensively in hemoglobinopathy patients, and good compliance with chelation in patients with β-thalassemia major improved their median survival from the teens to near normal. Deferoxamine is administered by daily continuous subcutaneous infusion (up to 40 mg/kg) over an 8- to 12-hour period. Local injection site complications are frequent and can be minimized by rotation of injection sites, addition of hydrocortisone to the infusion, antihistamines, or local measures. The potential ocular and auditory complications of deferoxamine mandate annual

Table 5-4 Iron chelation agents currently available for clinical use; properties and indications

Property	Deferoxamine	Deferiprone	Deferasirox
Usual dose	20–60 mg/kg/day	75–100 mg/kg/day	20–40 mg/kg/day
Route	Subcutaneous, intravenous >8–12 hours, >5 days/week	Oral 3 times daily	Oral Once daily
Half-life	20–30 minutes	3–4 hours	8–16 hours
Excretion	Urinary, fecal	Urinary	Fecal
Side effects*	Injection site reaction Potential ocular and/or otic toxicity†	Agranulocytosis (rare)	Renal insufficiency in up to 1/3‡ GI disturbance
Indications	Chronic IOL from transfusion-dependent anemias Acute iron intoxication	IOL in β-thalassemia major when DFO is contraindicated or inadequate	IOL from RBC transfusion in patients ≥2 years old (US) or ≥6 years old (Europe) IOL when DFO contraindicated or inadequate in: Other anemias Age 2–5 years (Europe)

Updated from Leitch HA, Vickars LM. *Hematology Am Soc Hematol Educ Program* 2009;2009:664–672, with permission from the American Society of Hematology.
DFO, deferoxamine; GI, gastrointestinal; IOL, iron overload; RBC, red blood cells.

*Monitoring as per product monograph for all agents.

†Yearly monitoring recommended for all.

‡Usually reversible or nonprogressive.

audiologic and ophthalmologic evaluations. Chronic deferoxamine therapy may be arduous, and suboptimal compliance often limits potential benefits. Preclinical studies aiming to increase the half-life of deferoxamine (from 5 to 20 minutes to 2 to 3 days) by binding it to a carrier molecule are in progress; this could potentially make treatment with this agent more attractive to patients.

Deferasirox was the first oral iron chelator to receive approval from the US Food and Drug Administration. In a prospective trial, 20 to 30 mg/kg of deferasirox daily (dispersible formulation; DF) reduced LIC, serum ferritin levels, and transaminases that were elevated at baseline prechelation. Adverse events related to the GI tract are frequent with deferasirox and may require dose reductions or other measures (published guidelines are available). Approximately one third of patients experience an increase in serum creatinine, which is usually reversible. Ocular and auditory disturbances are more frequent with deferoxamine at a ferritin level <1,000 ng/mL, and with deferasirox, this does not seem to be the case. The film-coated tablet formulation of deferasirox (FCT), has recently become available. The FCT has fewer GI side effects than the DF and is generally reported by patients as being more convenient. Because of differences in bioavailability, dosing of the FCT in mg/kg is 30% less than with the DF.

In the United States, the most recently approved oral iron chelator is deferiprone, which is dosed 3 times daily.

Side effects include GI upset, arthralgias, and elevated hepatic enzymes. Drug-induced neutropenia or agranulocytosis requires weekly monitoring of blood counts. Though typically not used for acquired anemias because of the potential for agranulocytosis, some small studies in this setting demonstrate safety and efficacy. Deferiprone appears to be particularly effective in reducing cardiac iron overload, which may be a function of its ability to cross the cell membrane. Experience with deferasirox (which also crosses cell membranes) for this indication is accumulating.

In some circumstances, intensification of chelation may be desirable. For example, it has been shown in β-thalassemia major that a ferritin level over 2,500 ng/mL portends inferior cardiac disease-free survival. Continuous infusions of deferoxamine or combination regimens should be considered in this circumstance at least until cardiac iron status and left ventricular ejection fraction are documented as negative and normal, respectively, and preferably until the ferritin level is consistently <2,500 ng/mL. For patients with documented cardiac iron loading or decreased left ventricular ejection fraction, intensive chelation may partially or fully reverse these complications and combination therapy with deferoxamine and deferiprone, or 24-hour infusions of deferoxamine, should be strongly considered. Combinations of deferoxamine and deferasirox are under study. Deferasirox as a single agent does improve cardiac iron-related abnormalities; however,

Table 5-5 Assessment of iron overload and common adverse events of chelators

Observation	Frequency	IOL assessment	AE monitoring
Iron intake rate	Each transfusion	✓	
Chelation dose and frequency	Every 3 months	✓	✓
Renal function*	As frequently as required		✓
Liver function	Every 3 months	✓	✓
Sequential serum ferritin, transferrin saturation†	Every 3 months	✓	
GTT, thyroid, calcium metabolism (BMD‡)	Yearly in adults	✓	
Liver iron (T2* MRI)§	At baseline where feasible and subsequently as clinically indicated	✓	
Cardiac function (echo, MRI, ecg)	At baseline, then as clinically indicated	✓	
Cardiac iron (T2* MRI)	For patients receiving >50 U RBC prior to ICT, or with CHF or arrhythmias	✓	
Slit lamp examination, audiometry	Yearly		✓

Reprinted from Leitch H, *Can Perspect Clin Hematol.* 2015;1:4–10, with permission from Canadian Perspectives in Clinical Hematology.

Ideal assessments are listed, and mandatory assessments are shown in boldface type.

AE, adverse event; BMD, bone mineral density; CHF, congestive heart failure; ecg, electrocardiogram; echo, echocardiogram; GTT, glucose tolerance test; ICT, iron chelation therapy; IOL, iron overload; MRI, magnetic resonance imaging; RBC, red blood cells; U, units.

*Creatinine should be measured at least every 2 weeks with each dose increase until stable.

†Transferrin saturation >80% may indicate the presence of oxidative stress (Sahlstedt L et al. *Br J Haematol.* 2001;113:836–838).

‡Based on early/suggestive data in transfusion dependent hemoglobinopathies [Ezzat H et al. *Blood.* 2012;120(21):abstract 3203].

§Up to 25% of hepatic IOL is underestimated by serum ferritin level (Gattermann N et al. EHA Annual Meeting 2013, poster 419).

intensification of the dose appropriate to the clinical situation may or may not be limited by side effects requiring dose interruptions and adjustments, and it is important to address iron-related cardiac complications in a timely manner. Until the treating physician has accumulated experience and a comfort level with the use of these agents, the expected side effects, and the monitoring required, input from a hematologist with this expertise should be considered.

Treatment of the acquired anemias is discussed in detail in Chapters 18 and 19, but a few words on chelation in these disorders may be appropriate here. Transfusions and iron chelation therapy are generally considered to be supportive care for acquired anemias. For MDS, the goal of active therapies such as erythropoiesis stimulating agents, immunomodulatory agents, or immunosuppressive therapies for lower-risk disease and hypomethylating agents for higher-risk disease is hematologic improvement, including an erythroid response and transfusion independence. The achievement of transfusion independence is widely recognized to improve survival and quality of life in these patients. Multiple nonrandomized studies of iron chelation therapy in MDS, and fewer in the less common conditions aplastic anemia and myelofibrosis, suggest su-

perior survival in patients receiving chelation compared to patients not receiving chelation. While these data are controversial, what is clear is that in multiple (but not all) studies of MDS, an erythroid response rate around 20% was seen with chelation, including the achievement of transfusion independence. Similar responses have been reported in myelofibrosis and may occur with more frequency in aplastic anemia. Patient characteristics predictive of erythroid response are currently unclear. The results of a randomized controlled trial of chelation in MDS are expected in late 2018.

Guidelines for chelation in MDS are extrapolated from experience with deferoxamine in hemoglobinopathies and, for example, suggest chelation once the ferritin level is >1,000 ng/mL or the transfusion burden >20 units of packed red blood cells. In the future, it may be more appropriate to institute (non-deferoxamine) chelation at lower doses to prevent iron overload rather than trying to rescue damaged tissue and being unable to increase the dose appropriately for the degree of iron overload because of side effects. This approach, however, should be confirmed to be safe and effective in clinical trials before it can be considered for routine practice.

KEY POINTS

- The absorption of iron by enterocytes and release of recycled iron from macrophages are tightly regulated by the interaction of the hormone hepcidin and iron transporter ferroportin.
- Iron overload may be due to hereditary or acquired causes, or to repeated blood transfusions.
- The HFE C282Y/C282Y is the most common genotype leading to clinical iron overload in hereditary hemochromatosis.
- The clinical penetrance of the HFE C282Y/C282Y genotype is probably <30%.
- Some clinical manifestations of hemochromatosis are reversible, but cirrhosis and the risk for hepatocellular carcinoma in cirrhotic patients are not.
- Population screening is controversial, but high-risk individuals (fasting transferrin saturation >45%, first-degree relative affected, Caucasian heritage) should be screened.
- Clinical manifestations of iron overload are similar regardless of etiology.
- Phlebotomy to remove excess iron is the primary treatment for conditions of iron overload not limited by anemia.
- Iron chelation therapy with deferoxamine or deferasirox is an option when phlebotomy is not possible. Monitoring, including regular creatinine levels and other chemistry, and annual audiologic and ophthalmologic examinations are required in individuals treated with these agents. Deferiprone is a more recently approved chelation agent for iron overload; monitoring for agranulocytosis is mandatory.

Heme synthesis

The first step in heme synthesis is condensation of glycine and succinyl CoA to form aminolevulinic acid (ALA) in the mitochondria, catalyzed by the enzyme ALA synthase (ALAS) (Figure 5–6). Six additional enzymes are involved in reactions that convert ALA to protoporphyrin, with some reactions occurring in the cytoplasm and others in the mitochondria. Specifically, ALA dehydratase (ALAD; in the cytoplasm) results in formation of porphobilinogen (PBG); PBG deaminase (PBGD; cytoplasm) results in formation of hydroxymethylbilane; uroporphyrinogen III synthase (UROS; cytoplasm) results in formation of uroporphyrinogen III; uroporphyrinogen decarboxylase (UROD; cytoplasm) results in formation of coproporphyrinogen III; coproporphyrinogen oxidase (CPO; mitochondria) results in formation of protoporphyrinogen; and protoporphyrinogen oxidase (PPO; mitochondria) results in formation of protoporphyrin IX. The final step in heme synthesis is the coupling of protoporphyrin to iron in the mitochondria, catalyzed by ferrochelatase. Although the steps are similar in erythroid cells and hepatocytes, control of heme production differs between tissues, mainly due to differences in the rate of ALA synthesis, which is coded by 2 different genes, *ALAS1* and *ALAS2*. In the liver, *ALAS1* is the rate-limiting enzyme, and its production is regulated by heme through negative feedback: it is downregulated by increased heme levels and upregulated by decreased heme. Since most heme synthesized in the liver goes toward the production of hepatic cytochromes, induction of cytochromes leads to the utilization of heme and therefore induction of *ALAS1*. The *ALAS1* gene and genes for certain cytochromes share upstream enhancer elements, which coordinate induction of these genes.

ALAS2 is constitutively expressed in erythroid cells and, in contrast to *ALAS1*, it is not negatively regulated by heme. Rather, *ALAS2* production is increased during erythroid differentiation via erythroid-specific transcription factors, like GATA1. Furthermore, *ALAS2* is post-transcriptionally regulated by iron, due to the presence of a 5' IRE in *ALAS2* mRNA (but not *ALAS1* mRNA). Increased iron availability leads to decreased IRP binding to 5' IRE and allows translation of *ALAS2* to proceed. Other enzymes in the heme synthetic pathway are also upregulated in the bone marrow during erythroid maturation to enhance hemoglobin synthesis. One practical implication of this difference between tissues is that heme can be used to treat an acute exacerbation of porphyria with hepatic manifestations such as acute intermittent porphyria (AIP), downregulating *ALAS1* activity. Conversely, steroids, chemicals, and stress can trigger exacerbations of hepatic

The porphyrias

Introduction

The porphyrias are disorders that result from enzymatic defects in the heme biosynthetic pathway. The word “porphyria” is derived from the Greek *porphuros*, or purple, which refers to the purple-red porphyrins that exhibit red fluorescence on exposure to ultraviolet light. Porphyrins complex with iron to form heme, a cofactor crucial for multiple biologic reactions and functions. Though heme synthesis occurs in mitochondria of virtually all cells, the 2 predominant areas are the bone marrow and liver. The bone marrow accounts for 85% of heme synthesis, as it is required for hemoglobin, and the liver for 15%, where it participates in the formation of several enzymes, most notably hepatic cytochromes.

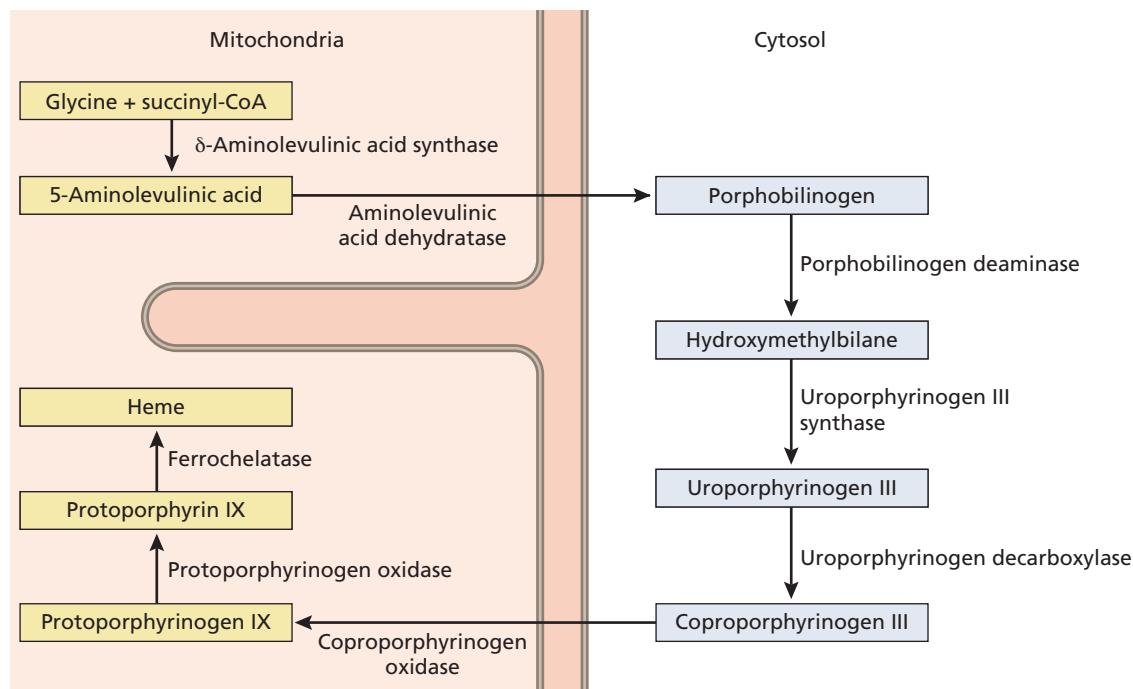


Figure 5-6 The heme biosynthetic pathway. Glycine and succinyl CoA are condensed to aminolevulinic acid (ALA) in the mitochondria, catalyzed by ALA synthase (ALAS). Six additional enzymes, localized in the cytoplasm or mitochondria, convert ALA to protoporphyrin. Protoporphyrin is coupled to iron to form heme. The rate of ALA synthesis is controlled in the liver by ALAS1, which is downregulated by increased heme and glucose levels and induced by steroids, chemicals and stress. In erythroid cells, the rate of ALA synthesis is limited by iron availability.

porphyrias by inducing ALAS1. Glucose suppresses ALAS1 expression, accounting for a higher incidence of clinical porphyria manifestation while fasting, and symptomatic response to glucose infusions.

Pathophysiology

The different porphyrias arise from a deficiency of different enzymes in the heme biosynthetic pathway (see Table 5-6), resulting in accumulation of porphyrins and their precursors in a pattern specific to the enzyme involved, which is reflected in clinical manifestations (see video file in online edition). During an acute exacerbation, the porphyrin precursors ALA and PBG are released in large amounts by the liver, and are neurotoxic, particularly for the autonomic and peripheral nervous systems. Although the blood-brain barrier protects the brain somewhat from these compounds, they may still cause vascular injury and brain edema. Characteristic skin symptoms develop from interaction of radiation with porphyrins that accumulate in the skin. Once the porphyrins absorb light, they emit energy and cause cell damage by peroxidation of lipid membranes, thus disrupting intracellular organelles. The principal site of photosensitivity is blood vessels of the

papillary dermis. Conventionally, symptomatic episodes in patients with porphyria have been referred to as acute attacks. As patients can go without symptoms for long periods of time and yet the underlying condition remains, we have referred to symptomatic episodes as exacerbations.

Inheritance

Most porphyrias are autosomal dominant with incomplete penetrance, though some types are recessive. Rarely, X-linked or complex patterns of inheritance such as compound heterozygotes may occur. The penetrance of porphyrias varies, with only about half of gene carriers demonstrating clinical manifestations.

Classification

Porphyrias are classified as acute or nonacute depending on presenting clinical features (Table 5-6). In acute porphyrias, accumulation of all porphyrin precursors proximal to the enzyme defect occurs. Precursors accumulate in large amounts due to decreased activity of PBGD, either due to genetic mutation, as in AIP, or by feedback inhibition in variegate porphyria (VP) or hereditary coproporphyria (HC). In nonacute porphyrias, accumulation of all porphyrins

Table 5-6 Classification of porphyrias

Type of porphyria	Inheritance pattern	Enzyme affected	Organs involved	Symptoms	Treatment	Comments
Acute porphyrias						
Acute intermittent porphyria	AD	Porphobilinogen deaminase/hydroxy-methylbilane synthase	NS, liver	NV	Glucose Hemin Supportive care* Liver transplant Gene therapy siRNA	No cutaneous symptoms Port wine-colored urine Common in Sweden
Porphyria variegata	AD	Protoporphyrinogen oxidase	NS, skin, liver	NV, cutaneous	Glucose Hemin Supportive care* Liver transplant Gene therapy	Common in South Africa
Hereditary coproporphyria	AD	Coproporphyrinogen oxidase	NS, skin, liver	NV, cutaneous	Glucose Hemin Supportive care*	Skin lesions occur but not common
ALA dehydratase porphyria	AR	ALA dehydratase	NS, liver	NV	Glucose Hemin Supportive care*	Very rare, chronic neuropathy ALA alone increased Late-onset type associated with MPN
Nonacute porphyrias						
Porphyria cutanea tarda	AD	Uroporphyrinogen decarboxylase	Skin, liver	Cutaneous	Control liver IOL Protect from sun/light exposure	Sporadic and familial forms exist
Erythropoietic protoporphyrinia	AR	Ferrochelatase	Skin, RBC, liver	Cutaneous	Sun/light protection Beta carotene Afamelanotide Measures for gallstones Liver+HSCT	Burning sensation in photosensitive areas Microcytic anemia Late onset type associated with MDS
Congenital erythropoietic porphyria	AR	Uroporphyrinogen III synthase	Skin, RBC	Cutaneous, hemolytic anemia	Suppress erythropoiesis HSCT	Erythrodontia (teeth fluoresce) Red fluorescent urine Bone changes
Hepatoerythropoietic porphyria	AR	Uroporphyrinogen decarboxylase	Skin, RBC, liver	Cutaneous, hemolytic anemia	Sun/light protection	Lab results similar to PCT Red urine

In all conditions which involve the liver, chronic liver failure and hepatocellular carcinoma may develop.

*See Table 5-7 for supportive care measures.

AD, autosomal dominant; AR, autosomal recessive; HSCT, hematopoietic stem cell transplantation; IOL, iron overload; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NS, nervous system; NV, neurovisceral; PCT, porphyria cutanea tarda; RBC, red blood cells; siRNA, small interfering RNA.

formed before the enzyme defect occurs, but there is no increase in porphyrin precursors, possibly because of a compensatory increase in activity of PBGD. Additional classification as hepatic or erythropoietic porphyria is based on the organ in which accumulation of porphyrins and their precursors primarily occurs.

Porphyria in the 21st century

A survey of 108 patients with porphyria from the American porphyria consortium showed that most were females, with around half not having a known affected parent. Approximately 80% with AIP, and 77% and 100% of patients with HC and VP, respectively, reported first symptoms in the second to fourth decades. Symptoms of AIP were intermittent but frequent in about half, present only during acute exacerbations in about one quarter and constant in 18%. Triggers for AIP episodes included medication (37%), diet (22%), surgery (16%), and environmental toxins (7%). Abdominal pain, nausea and vomiting, weakness and constipation were the most frequent presenting symptoms of acute exacerbations. Chronicity of AIP included the development of peripheral neuropathy, hypertension, seizures, psychiatric conditions, chronic renal disease, and hepatic cirrhosis. Oral contraceptives worsened symptoms of porphyria in one-third of women. Pregnancy was uneventful in 59 out of 60 cases, with the delivery of healthy newborns.

The majority of those who received intravenous heme (hematin) for AIP found it very effective in improving symptoms, and also successful in preventing acute attacks. In contrast, among those who received opiates, less than half found them helpful.

Genetic analysis can detect mutations in AIP, HC, and VP, although no significant associations between these mutations and clinical symptoms or laboratory abnormalities were reported.

Acute porphyrias

Four porphyrias present with acute features, including the most common—AIP, HC, VP—and the rare δ -ALA dehydratase porphyria.

Acute intermittent porphyria

AIP, also known as Swedish porphyria, results from deficient activity of PBGD. It affects about 1 in 75,000 people of European descent, except in northern Sweden, where 1 in 1,000 are affected. AIP does not have skin manifestations. Mutations underlying AIP typically reduce the activity of PBGD by around 50%, which does not always result in symptoms unless there is induction of the rate-limiting hepatic enzyme ALAS1, which can occur as a result of some medications, endocrine factors, and reduced calorie

intake. High levels of PBG contribute to the reddish or port wine-colored urine seen in AIP. Erythrocyte PBGD activity is decreased in most patients, although about 5% have de novo mutations only in hepatocytes, in which case detection of the PBGD mutation confirms the diagnosis.

Other acute porphyrias

VP, which occurs as a result of deficiency of protoporphyrinogen oxidase, and HC, a result of deficiency of coproporphyrinogen oxidase, present with cutaneous photosensitivity and neurovisceral symptoms. Cutaneous manifestations are from an accumulation of photosensitizing porphyrins, which does not occur in AIP because the enzyme block is upstream of porphyrin production. Skin lesions develop in about 60% of patients with VP and 5% with HC, usually many days after sun exposure, and typically on the back of the hands, with fragility, blistering, and scarring occurring. VP is predominant in South Africa, where a characteristic mutation, Arg59Tryp, occurs. VP and HC, like AIP, usually have autosomal dominant inheritance. Recessive forms of AIP, VP, and HC have also been described in children with neurological symptoms and developmental delay. δ -ALA dehydratase porphyria (ALAD), also known as doss or plumboporphyria, is the only acute porphyria with autosomal recessive inheritance. In contrast to the other acute porphyrias, ALA but not PBG is increased in the urine. Marked deficiency of ALAD in the absence of lead poisoning suggests the diagnosis in most. Chronic neuropathy can develop. A late-onset type may be seen in association with myeloproliferative neoplasms.

Clinical features of acute porphyria

The predominant symptoms of acute porphyrias are neurovisceral. Exacerbations can begin with restlessness and insomnia and may progress rapidly. A typical presentation is abdominal pain, vomiting, constipation, and bladder paresis. Pain in the back or extremities is common. Features which differentiate acute porphyrias from an acute abdomen include poor localization, absence of peritoneal signs or fever, and absence of leukocytosis. The pathogenesis of pain is not well understood, though autonomic neuropathy, disturbances in smooth muscle function, intestinal angina, and lack of nitric oxide have all been proposed. Since similar pain episodes are also seen in hereditary tyrosinemia and lead poisoning, all of which perturb heme synthesis, delta-aminolevulinic acid (δ -ALA) has been suggested as the cause of pain and the efficacy of hemin infusion may be due to its inhibition of the enzyme that catalyzes δ -ALA formation. The most common clinical signs are tachycardia and hypertension, suggestive of autonomic dysfunction, which can lead to arrhythmias and even cardiac arrest.

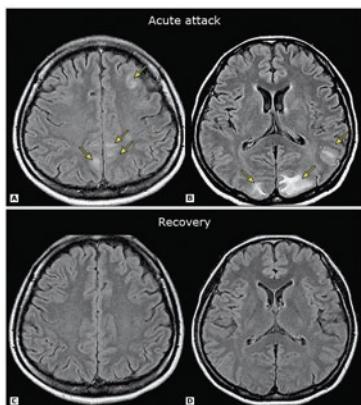


Figure 5-7 Brain MRI images showing posterior reversible encephalopathy syndrome in acute intermittent porphyria (AIP), in which central nervous system involvement may develop.

The mechanism of neural damage in AIP is not well understood but might involve damage to neurons from porphyrin precursors. From Kuo HC et al. *Eur Neurol*. 2011;66(5):247–252, with permission.

Peripheral neuropathy occurs in about 40% of acute porphyria exacerbations, usually following the onset of abdominal symptoms. Motor neuropathy is predominant and must be differentiated from Guillain-Barré syndrome (GBS); mental disturbances, recurrent episodes, and abdominal symptoms are unusual with GBS. Proximal muscles are predominantly affected, with upper-limb involvement in 50%. Sensory neuropathy, when it occurs, has a bathing-trunk distribution, while cranial nerve involvement generally develops later. Respiratory muscle weakness and respiratory failure may develop. Central nervous system involvement, such as encephalopathy, can develop and cerebrospinal fluid examination is often normal. Seizures also may occur, often associated with severe hyponatremia. Metabolic disturbance is due to a syndrome of inappropriate antidiuretic hormone secretion, gastrointestinal loss, and volume depletion. Imaging demonstrates changes consistent with the posterior reversible encephalopathy syndrome of acute hypertensive episodes (Figure 5-7). The mechanism of neural damage in acute porphyrias is not well understood, although vasospasm resulting from decreased nitrous oxide production by nitrous oxide synthase, a hemeoprotein, or neurotoxicity from porphyrin precursors taken up into neurons have been suggested. That neurologic manifestations occur because of heme production by the liver is supported by dramatic responses to liver transplantation. Psychiatric disturbances—including depression, hallucinations, and even frank psychosis—may be a feature of acute porphyria. Many porphyria patients are described as having a psychiatric disorder. Nonspecific symptoms such as fatigue are also common, with up to 50% affected.

Acute porphyrias commonly are associated with abnormalities in liver function tests and have a significantly higher risk of advanced liver disease and hepatocellular carcinoma. Because serum α -fetoprotein may not always be raised, regular screening using imaging is advisable in adult patients. Chronic renal impairment may develop because of hypertension, although repeated vasospasms during recurrent attacks have also been implicated.

Triggers of acute porphyrias

Many medications can precipitate exacerbations of acute porphyria. Mechanisms include induction of hepatic cytochrome P450 and ALAS-1, and inhibition of other enzymes of heme synthesis. Safe and unsafe medications are listed at <http://www.porphyrnia-europe.com> and <http://www.drugs-porphyrnia.org>. Some medications are definitely contraindicated; however, many others are only potentially dangerous and the risk versus benefit of the use of these medications should be considered on a case-by-case basis.

Acute episodes are more common in women during the second to fourth decades, occurring rarely before puberty and after menopause. Menstrual cycles are a common precipitant, with recurrent episodes described typically in the late luteal phase, as progesterone is implicated in increased heme catabolism. Although oral contraceptives may aggravate exacerbations, postmenopausal hormone replacement therapy does not seem to be a trigger. Other common aggravating factors are fasting, alcohol intake (which induces or inhibits many enzymes in the heme biosynthetic pathway), infection, and several medications.

Diagnosis of acute porphyria

An index of suspicion for acute porphyria must be maintained, as delayed treatment may result in serious consequences such as neurologic damage and even death. It is important to note that although abdominal pain typically occurs after exposure to a precipitating factor, approximately one tenth of patients may not have any abdominal symptoms.

The first step in diagnosis of acute porphyria is to correctly collect 24-hour urine to obtain evidence that there is an ongoing episode of AIP, VP, HC, or ALAD porphyria; and second is to determine the acute porphyria subtype. For this, a review of the 24-hour urine, stool, and selected blood tests (eg, red cell enzyme determinations for PBGD), must be done. The clinical status of the patient is important in determining approach, because if critically ill, more rapid qualitative screening tests should be obtained; however, these may not be readily available, so empiric therapy might have to be started. Clinicians must understand the ordering system of the reference laboratory to ensure that

appropriate tests are ordered and collected correctly to make a diagnosis. For example, some reference laboratories include testing for PBG or ALA in a 24-hour urine study, whereas with others these must be ordered separately. If one is seeing a suspected acute porphyria patient for the first time, 24-hour determinations of both PBG and ALA should be done to exclude ALAD porphyria.

A common clinical circumstance is an elevation of coproporphyrins in the urine of patients suspected of having a neuropathic porphyria. These patients usually have secondary coproporphyrinuria, with the critical diagnostic point being that they have normal PBG levels while symptomatic—a finding that excludes neuropathic porphyria if the urine was collected correctly. Another common outpatient circumstance is that all prior 24-hour urine tests were collected when asymptomatic. Since PBG levels can normalize between exacerbations, the patient should be instructed to collect a 24-hour urine sample during clinical symptoms. True exacerbations of acute neuropathic porphyria are diagnosed easily and have abnormally high levels of PBG or ALA. If a patient has been evaluated for long periods of time, but always with indeterminate results, acute neuropathic porphyria is less likely.

Once the recognition of an acute porphyria episode has been made, confirmatory tests to determine the subtype of acute porphyria should be done. Patients with VP and HC have characteristic 24-hour stool findings even between attacks. Biochemical confirmation of the type of acute porphyria can be made by measuring erythrocyte PBG deaminase levels (AIP) and urine, plasma, and fecal porphyrin levels by high-performance liquid chromatography or fluorometric tests. DNA analysis or enzyme measurements are useful for family members if a mutation is confirmed in the index case. Genetic counselors should be involved for familial studies.

The differential diagnosis of acute porphyria includes lead toxicity, where abdominal pain and neuropathy can coexist, and paroxysmal nocturnal hemoglobinuria, where abdominal pain and discolored urine occur in the absence of peripheral neuropathy (but in this case anemia occurs). The combination of peripheral neuropathy with central nervous system involvement is unusual in other conditions and should alert the clinician to the possibility of porphyria. Hereditary tyrosinemia type 1, which develops as a result of accumulation of succinyl acetone, an inhibitor of ALAD, can present in children with symptoms resembling acute porphyria.

Treatment of acute porphyria

Patients who present with acute porphyria should be hospitalized. All contraindicated medications should be

stopped. A multidisciplinary approach should be taken because the clinical manifestations encompass multiple organ systems. Mild episodes, without signs and symptoms such as severe abdominal pain, neuropathy, and hyponatremia, may be treated initially with high carbohydrate intake of 2,000 kcal/24 hours orally or via a nasogastric tube. If this cannot be tolerated, intravenous 10% dextrose should be given targeting at least 300 g/day glucose, but precaution should be taken to avoid larger quantities, which may lead to hyponatremia. Opioids and phenothiazines can be given if necessary. Beta blockers can be used to treat tachycardia and hypertension.

Severe episodes require treatment with intravenous infusions of hemin, which binds to hemopexin and albumin in the plasma and is taken up by the liver, where it suppresses ALAS. This agent should be started early for better clinical outcome. The standard regimen is 1 to 4 mg/kg once daily of heme, from lyophilized hematin, reconstituted with human albumin in order to avoid thrombophlebitis (Panhematin; Recordati Rare Diseases Inc, Lebanon, NJ), and infused daily for 3 to 14 days, or heme arginate (Orphan Europe), infused daily for 4 days. Hematin is safe in renal impairment. Adverse effects include fever, hemolysis, and before reconstitution with albumin was employed, phlebitis. Response to therapy often occurs within 1 to 2 days, particularly if commenced early. The full 4-day course of treatment should be completed.

Careful monitoring is advisable for early detection of complications (Table 5-7). At hospital discharge, advice should be provided for measures to prevent future exacerbations (Table 5-8). Because oral contraceptives are common precipitants, gonadotropin-releasing hormone analogues can be used as alternatives given during the first few days of the menstrual cycle, but regular gynecologic assessment and bone density measurements are necessary. Although pregnancy increases levels of progesterone, women who have had acute porphyria should not be advised against pregnancy but rather should be managed in a specialist center that has experience in dealing with porphyria. Heme arginate is safe in pregnancy and repeated use does not affect pregnancy outcome.

About 10% of patients with acute porphyria have recurrent exacerbations. Once-weekly hematin infusions have been suggested as prophylaxis. However, this may cause venous thrombosis, necessitating central venous access and resulting in iron overload.

Allogeneic liver transplantation has been performed in AIP and VP with success. After transplantation, urinary ALA and PBG levels returned to normal within 24 hours. This, however, should be considered only in those who experience recurrent severe attacks. Gene

Table 5-7 Supportive measures and monitoring in acute porphyria

Supportive measures
• Nutritional support: oral, nasogastric, or intravenous
• Pain relief: opiates
• Volume depletion: intravenous fluids
• Insomnia and restlessness: chloral hydrate or low doses of short-acting benzodiazepines
• Nausea and vomiting: chlorpromazine and prochlorperazine
• Tachycardia and hypertension: beta-blockers with care (hypovolemia)
• Seizure prophylaxis, particularly if hyponatremia coexists, and seizure control: gabapentin or vigabatrin; benzodiazepines may be safe
• Anesthesia if required: nitrous oxide, ether, halothane, or propofol
• Muscle relaxants: suxamethonium
• Bladder paresis: catheterization
Monitor
• Serum electrolytes, particularly sodium and magnesium
• Renal and liver tests
• Vital capacity: consider intensive care management if deteriorating
• Neurologic status
• Bladder distension

Table 5-8 General and follow-up measures for acute porphyria

Counsel
• Alcohol avoidance
• Smoking cessation
• Information about safe and unsafe medications in porphyria
• Avoidance of oral contraceptives
• Maintain adequate nutrition
• Arrange for medical bracelets
• Psychological input for depression
• Genetic counseling for families
• Photoprotection*
• Avoidance of sunlight exposure and skin trauma*
Follow-up
• For liver problems, especially chronic liver failure and hepatocellular carcinoma
• Those with chronic hypertension require close follow-up
• Management of chronic pain
• Management of chronic mental health issues

*For porphyrias with cutaneous manifestations only.

therapy with adeno-associated virus vector delivering the PBGD gene, and enzyme replacement with recombinant human PBGD, have been attempted. Small interfering RNA (siRNA) therapy to decrease production of ALA by decreasing ALAS1 is under evaluation.

Nonacute (cutaneous) porphyrias

These differ from acute porphyrias mainly by the absence of neurological symptoms.

Porphyria cutanea tarda

Porphyria cutanea tarda (PCT) is the most common non-acute porphyria, it is also referred to as a cutaneous porphyria. PCT can be either sporadic (type 1) or familial (type 2). The sporadic form accounts for 80% of cases. In the absence of any mutations, clinical symptoms develop when enzyme activity decreases to less than 20% of normal. In the familial variety, which accounts for 20% of cases, patients are heterozygous for mutations in uroporphyrinogen decarboxylase (UROD). Since patients have 50% enzyme activity, many are asymptomatic unless other precipitating factors occur. Hepatitis C, HIV, and mutations in the hemochromatosis gene can contribute to pathophysiology by increasing liver iron, which via ROS results in the inhibition of UROD.

PCT usually presents in adults and is characterized by bullous cutaneous lesions, which often start as erythema and become confluent to form blisters, most often observed on the backs of the hands and other light-exposed areas (Figure 5-8). When blisters rupture, they can cause scarring. Small white papules (milia) are common in the same areas. Hyperpigmentation and increased hair growth, particularly on the face, can cause disfigurement. Occasionally, the skin in sun-exposed areas becomes severely thickened, termed pseudoscleroderma. Skin symptoms show seasonal variations with more symptoms in the summer and autumn. Similar to other porphyrias, there is excretion of colored/fluorescent porphyrins in the urine (Figure 5-8). Liver dysfunction is common and can vary from mild impairment to cirrhosis. The incidence of hepatocellular carcinoma is higher in these patients. Rare ocular complications have been reported.

Plasma porphyrin analysis is the best initial test for PCT, with very high levels of isocoproporphyrin noted in the feces. In addition to avoiding precipitating factors such as alcohol and iron supplements, phlebotomy to reduce hepatic iron is the cornerstone of treatment. Because iron overload is generally not marked, the target ferritin can be readily achieved. The plasma porphyrin level can be followed as phlebotomies are done, with expected control of skin lesions when elevations of plasma porphyrins are no

longer detected. Iron chelation therapy may be considered if a patient cannot tolerate phlebotomy. Low-dose chloroquine (125 mg twice weekly) can mobilize liver porphyrins to be excreted in the urine. This may be used in conjunction with or as an alternative to phlebotomy; however, caution should be used in those with severe liver impairment as chloroquine may cause hepatitis. Underlying diseases such as hepatitis C should be treated and opaque sunscreens containing zinc oxide should be used.

Pseudoporphyria is a bullous disorder with clinical and histologic features similar to those of PCT but without the characteristic biochemical abnormalities. It originally was observed as skin lesions in patients with renal failure, so-called bullous dermatosis of hemodialysis. Several medications have been associated with pseudoporphyria, including naproxen, nalidixic acid, dapsone, amiodarone, and diuretics. It also may occur in individuals using tanning beds. Clinical features of pseudoporphyria are identical to PCT except that the legs, upper chest, or face may also be involved. In contrast to PCT, however, hypertrichosis and hyperpigmentation usually are not seen. Treatment involves discontinuation of suspected exacerbating factors and sun protection. Hemodialysis-associated pseudoporphyria has been reported to respond to treatment with the antioxidant *N*-acetylcysteine.

Erythropoietic protoporphyrinia

Erythropoietic protoporphyrinia (EP), the most common porphyria in children, results from mutations in the ferrochelatase gene and is usually inherited in autosomal recessive fashion. In EP, skin lesions begin in early childhood. A characteristic symptom is a burning sensation which develops very quickly in sun-exposed areas. These may turn erythematous but rarely vesiculate. Chronic skin changes can develop although severe scarring, hyperpigmentation, and hirsutism are rare. Some patients may have a microcytic, hypochromic anemia. Late-onset EP has been described in association with MDS. Another unusual feature of EP is the development of gallstones in the absence of hemolysis, probably due to excess protoporphyrin decreasing bile flow. Liver disease is common but typically develops after age 30. The diagnosis of EP is made by measuring total and fractionated porphyrins and protoporphyrin.

Management of EP includes protection from sunlight using special clothes, opaque sunscreens, or ultraviolet-B phototherapy. The antioxidant oral β -carotene, given at 75 to 200 mg/day, may alleviate solar sensitivity, but can cause yellowish skin discoloration. Melanocyte-stimulating hormone analogues, which darken the skin, have also been tried (afamelanotide). Because biochemical signs of iron de-



Figure 5-8 Porphyria cutanea tarda (PCT) results from decreased activity of uroporphyrinogen decarboxylase (UROD). (A–B) Sun-exposed hands of a PCT patient showing areas of atrophy and scarring secondary to accumulation of porphyrin precursors and exposure to ultraviolet light. Once the porphyrin precursors absorb light, they emit energy and cause cell damage by peroxidation of lipid membranes, thus disrupting intracellular organelles. (C) Urine from a symptomatic PCT patient and a healthy control in daylight (left) and under ultraviolet light (right). The PCT urine has an orange-red color in daylight that fluoresces red under ultraviolet light. From Balwani M, Desnick RJ. *Hematology Am Soc Hematol Educ Program*. 2012;2012:19–27, with permission.

ficiency and low vitamin D levels are frequent findings, vitamin replacement and close monitoring for anemia is necessary. Interruption of enterohepatic circulation of protoporphyrin with cholestyramine or activated charcoal may prevent liver damage. Although liver transplantation has been attempted in EP, its success is limited because of continued production of protoporphyrin by the bone marrow. Sequential liver and bone marrow transplantation has also been described. During surgery, modification of lighting is necessary to limit organ injury.

An X-linked form of EP due to gain-of-function mutations in the *ALAS2* gene has recently been described. In this condition, there is no ferrochelatase deficiency, but large amounts of protoporphyrin accumulate in erythrocytes, much of which is bound to zinc. This is in contrast to previously described mutations in the *ALAS2* gene, which are loss-of-function and cause X-linked sideroblastic anemia.

Congenital erythropoietic porphyria

Congenital erythropoietic porphyria (CEP), also known as Günther disease, was the first porphyria to be described. It is unique among nonacute (cutaneous) porphyrias in being an autosomal-recessive disorder and is due to deficient activity of uroporphyrinogen III synthase. The severe cutaneous photosensitivity in CEP begins in early infancy. In addition to blistering, the skin is extremely friable and becomes easily infected. Repeated infections, hypertricho-

sis, and bone resorption in CEP lead to severe disfigurement. Because of deposition of excess porphyrins in the teeth, they become reddish brown (erythrodontia), and fluoresce in ultraviolet light (Figure 5-9). Corneal scarring and keratitis cause ocular problems. An excess of red cell protoporphyrins, which are needle-like inclusions on blood smear examination, can cause nonimmune hemolysis and splenomegaly. In some cases, this may develop in utero and manifest as hydrops fetalis.

Early diagnosis of CEP is necessary to avoid phototherapy for neonatal jaundice, and red fluorescent urine in diapers is suggestive. The management of CEP is based on suppressing erythropoiesis; iron deficiency achieves this and underscores the link between iron and heme metabolism. Sunlight protection and avoidance of skin trauma are also important. Bone marrow transplantation is effective, while splenectomy and hypertransfusion have shown no benefit.

Hepatoerythropoietic porphyria

This rare condition is caused by homozygous or compound heterozygous deficiency of uroporphyrinogen decarboxylase. Hepatoerythropoietic porphyria presents in infancy or childhood and has clinical characteristics similar to CEP, with red urine, skin lesions, and scarring, and hemolytic anemia and splenomegaly may also develop. Laboratory findings are similar to PCT, however, and treatment is based on sunlight avoidance, without response to phlebotomy.

Figure 5-9 Erythrodontia in congenital erythropoietic porphyria, which results from deficient activity of uroporphyrinogen III synthase. Excess porphyrins are deposited in the teeth, which become reddish brown (erythrodontia) and fluoresce in ultraviolet light. From Balwani M, Desnick RJ. *Hematology Am Soc Hematol Educ Program*. 2012;2012:19–27, with permission.





KEY POINTS

- The most common porphyrias are acute intermittent porphyria (AIP), an acute porphyria without skin findings, and porphyria cutanea tarda (PCT), a nonacute porphyria with primarily cutaneous manifestations.
- With acute exacerbations of porphyria, levels of the substrate PBG (or rarely, ALA) are increased by several logs. Mild elevations are not diagnostic of porphyria.
- It is important to understand and follow reference laboratory instructions for the correct collection and handling of specimens in order to make a diagnosis of porphyria.
- Many more patients carry a diagnosis of porphyria than actually have the disease.
- Many mutations are described in the PBG gene. Having a genetic defect alone does not equate with disease because of highly varied penetrance.

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6

Acquired underproduction anemias

MOHANDAS NARLA AND JACQUELYN M. POWERS

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Introduction

Erythropoiesis is the process by which hematopoietic stem cells divide, differentiate, and mature into enucleated red blood cells (RBCs). The earliest identifiable erythroid progenitor is the burst-forming unit-erythroid, which is defined functionally by its ability *in vitro* to form large “bursts” of erythroblast colonies of various sizes after approximately 2 weeks in semisolid media. Each burst-forming unit-erythroid can generate between 1,000 to 10,000 erythroblasts. The next defined stage is the colony-forming unit-erythroid (CFU-E), which under low concentrations of erythropoietin (EPO) give rise to 100 to 200 well-hemoglobinized erythroblasts after approximately 1 week in culture. The erythroid stages subsequent to CFU-E (proerythroblast to basophilic erythroblast to polychromatic to orthochromatic erythroblast) are defined by their light microscopic appearance on marrow aspirate slides. The pyknotic erythroblast (nucleated red blood cell) undergoes enucleation to produce a reticulocyte, which spends 1 to 2 days in the marrow followed by 1 to 2 days in the peripheral blood, in which the RNA is completely lost and the mitochondria are degraded and the mature red cell results.

EPO is the primary cytokine that controls erythropoiesis and acts on erythroid progenitors in the stages of CFU-E to the earliest basophilic erythroblasts. It takes approximately 7 days for a CFU-E to differentiate into a reticulocyte and clinically, this corresponds to the absolute reticulocyte count increase of approximately 7 days after EPO signaling (eg, following acute hemorrhage). EPO is produced primarily in the kidney, and its mRNA expression is increased by hypoxia via the transcription factor hypoxia-inducible factor. Chuvash polycythemia, an autosomal recessive form of erythrocytosis endemic in the Chuvash Republic of the Russian Federation, results from constitutive EPO signaling due to mutations in a protein required for the destruction of hypoxia-inducible factor under normoxia conditions. Dimerization of the EPO receptor activates receptor-associated Janus kinase 2, a kinase that is mutated in the majority of patients with polycythemia vera (see Chapter 16). This activation event initiates a sequence of signaling reactions that prevents apoptosis and stimulates proliferation and maturation of erythroid cells.

Heme is a complex of ferrous iron and protoporphyrin IX (PPIX). There are 8 enzymes in the mammalian heme synthetic pathway (Figure 6-1). The

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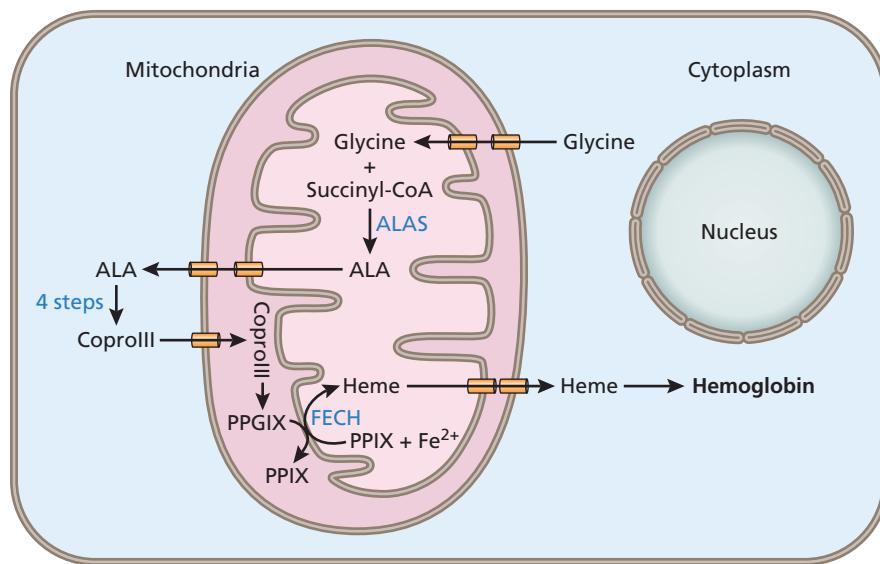


Figure 6-1 Heme synthesis. ALA, 5-aminolevulinic acid; CoproIII, coproporphyrinogen III; PPGIX, protoporphyrinogen IX; PPIX, protoporphyrin IX; ALAS, 5-aminolevulinic acid synthase; FECH, ferrochelatase.

first step occurs within the mitochondria where 5-aminolevulinate synthase (ALA-S2), along with vitamin B₆, catalyzes the condensation of glycine and succinyl coenzyme A (CoA) to yield δ-aminolevulinic acid (ALA). This is the rate-limiting step in heme production and is regulated by iron availability in erythroid cells. ALA is transported to the cytosol, where 4 additional enzymatic reactions occur, producing coproporphyrinogen III (Copro III), which is transported back into the mitochondria for the remaining 3 steps in the pathway. The final step, catalyzed by the enzyme ferrochelatase (FECH), incorporates iron into PPIX.

In adults, approximately 200 billion erythrocytes are produced each day to replace senescent red cells that are removed from circulation. This requires bone marrow stem cells, iron, cytokines (including EPO), vitamins, and a suitable marrow microenvironment. Deficiency or unavailability of any of these key components results in decreased RBC production and anemia.

We define underproduction anemias clinically by the presence of anemia and a corrected reticulocyte count [(reticulocyte percent × patient's hematocrit)/normal hematocrit] of approximately <2%, which indicates an inappropriately low response by the marrow to the degree of anemia. The acquired and congenital (reviewed elsewhere) underproduction anemias can be further grouped by RBC size—that is, mean corpuscular volume (MCV)—into microcytic (eg, iron deficiency anemia

[IDA], thalassemia), normocytic (eg, anemia of inflammation, anemia associated with chronic kidney disease), and macrocytic (eg, megaloblastic anemias, acquired pure red cell aplasia, and myelodysplastic syndromes [MDS]). The normal ranges of MCV vary by age, gender, and ancestry, and physicians should take into consideration that the same reference standards for hemoglobin and MCV do not apply to all patients. In persons of African ancestry, for example, some of this variability may reflect the higher prevalence of alpha thalassemia. A number of other acquired anemias with low corrected reticulocyte counts are not routinely categorized by cell size, but are often normocytic. These conditions can be complicated by multiple pathophysiologies contributing to suppressed RBC production and are discussed in separate sections within this chapter (ie, “Anemia of cancer,” “Myelophthistic anemia,” “Anemia of malnutrition,” “Anemias associated with endocrine disorders and pregnancy,” “Anemia of the elderly,” and “Anemia associated with HIV infection”). This chapter focuses only on the acquired underproduction anemias (see Chapter 16 for congenital underproduction anemias). A variety of primary hematopoietic disorders can affect the bone marrow and lead to acquired underproduction anemia as well other cytopenias. Detailed discussion of these entities is included elsewhere (eg, aplastic anemia, acute leukemia, and MDS). An outline of the acquired underproduction anemias covered in this chapter is depicted in Table 6-1.

Table 6-1 Selected acquired underproduction anemias reviewed in this chapter

Microcytic*	Iron deficiency anemia
Normocytic	Anemia of inflammation (~30% are microcytic)
	Anemia associated with chronic kidney disease
Macrocytic	Megaloblastic anemia (vitamin B ₁₂ and folate deficiencies)
	Acquired pure red cell aplasia
	Anemia associated with liver disease
	Acquired sideroblastic anemias (often macrocytic) [†]
Other	Anemia of cancer
	Myelophthisic anemias
	Anemia from malnutrition/anorexia nervosa
	Anemia associated with endocrine disorders
	Anemia associated with pregnancy
	Anemia of the elderly
	Anemia associated with HIV infection

*If we consider all underproduction microcytic anemias (not just those that are acquired), one can think of these broadly as caused by heme (iron, many congenital sideroblastic anemias) or globin (thalassemia) deficiency.

[†]Many (but not all) congenital sideroblastic anemias are microcytic.

Microcytic anemias

Iron deficiency anemia

CLINICAL CASE

A 72-year-old man presents to his primary care provider complaining of increasing dyspnea on exertion and fatigue. Laboratory evaluation reveals a microcytic anemia with a hemoglobin of 7.4 g/dL, MCV of 74 fL, and reticulocyte count of 1%. White blood cell count is normal, and the platelet count is slightly elevated at 502,000/mL. Iron studies reveal a low serum iron, elevated total iron-binding capacity (TIBC), and a markedly reduced ferritin of 9 µg/L. A workup for gastrointestinal (GI) bleeding, including upper and lower endoscopy, reveals angiodysplastic lesions of the large bowel. Intravenous ferric carboxymaltose is administered with good clinical response.

Background

Iron deficiency (defined by a low serum ferritin) is the most common cause of anemia worldwide, affecting over 1 billion people, predominantly women and children. Data from the U.S. National Health and Nutrition Examination Survey (NHANES) from 2003 to 2010 found that iron deficiency affected approximately 15% of toddlers, 11% of

nonpregnant adolescent girls, and 9% of adult women (age 20 to 49 years).

Iron homeostasis

Consideration of total iron body content and trafficking (see Figure 5-1) is helpful when calculating iron requirements needed to correct a patient's iron deficit. The vast majority of the body's iron is contained in hemoglobin within erythroid cells, of which approximately 25 mg is recycled each day. Senescent RBCs are phagocytosed by reticuloendothelial macrophages, which degrade hemoglobin and export the released iron into the plasma where it binds transferrin. Transferrin-bound iron is then delivered to the bone marrow to support new RBC production or to the liver for storage as ferritin (~1 g in men and ~300 to 600 mg in menstruating women) or other sites. One to two milligrams of new iron enters the body each day from dietary intake and absorption, to replace that same amount of iron lost daily via normal sloughing of skin and intestinal cells, as well as menstrual blood loss in women.

Intestinal iron absorption and mobilization of storage iron from macrophages and hepatocytes are controlled by both a store regulator and an erythroid regulator. The store regulator maintains the body's normal iron requirements and stores; the erythroid regulator maintains iron supply to the erythron regardless of the body's iron balance. Hepcidin, a key regulator of iron metabolism, is likely the final mediator of both the store regulator and the erythroid regulator. Ferroportin is the transmembrane iron export protein found on enterocytes and macrophages. Hepcidin acts by binding ferroportin, leading to its degradation, and thus inhibits both dietary iron absorption and release of iron from macrophages. Recently identified erythropherrone, a protein hormone produced by erythroblasts, inhibits the action of hepcidin and thereby increases the amount of iron available for hemoglobin synthesis in times of stress erythropoiesis. Systemic and cellular iron homeostasis are described in detail in Chapter 5.

Intestinal iron absorption depends on: dietary iron amount, bioavailability, and physiological requirements. A typical Western diet contains approximately 10 to 20 mg of iron (roughly 6 mg of iron per 1,000 calories), mostly as inorganic iron (cereals and legumes), and heme iron (red meats, fish, poultry). Inorganic iron is absorbed less readily than heme iron. In iron-replete patients, approximately 10% of inorganic iron vs 30% of heme iron is absorbed. Reviewing forms of iron consumed, other factors that affect iron absorption (Table 6-2), and the iron content of foods (Table 6-3) are useful when providing dietary counseling to iron-deficient patients.

Table 6-2 Factors that affect dietary iron absorption

Inhibit absorption	Enhance absorption
Calcium-rich foods	Ascorbic acid
Tannins in tea and coffee	Heme iron; ferrous iron (Fe^{2+})
Phytates in cereals	Legumes (remove phytates)

Table 6-3 Iron content of selected foods

Food	Milligrams per serving	Percent DV*
Select breakfast cereals, fortified with iron, 1 serving	18	100
White beans, canned, 1 cup	8	44
Dark chocolate, 45%–69% cacao solids, 3 ounces	7	39
Oysters, 6 medium	6	33
Beef liver, pan fried, 3 ounces	5	28
Blackstrap molasses, 1 tablespoon	3.5	19
Lentils, boiled and drained, $\frac{1}{2}$ cup	3	17
Spinach, boiled and drained, $\frac{1}{2}$ cup	3	17
Firm tofu, $\frac{1}{2}$ cup	3	17
Kidney beans, canned, $\frac{1}{2}$ cup	2	11
Chickpeas, boiled and drained, $\frac{1}{2}$ cup	2	11
Tomatoes, stewed and canned, $\frac{1}{2}$ cup	2	11
Beef, 3 ounces cooked	2	11
Baked potato, medium sized	2	11
Cashew nuts, oil roasted, 1 ounce	2	11
Chicken, dark meat, 3 ounces cooked	1	6

*DV = daily value recommended by the U.S. Food and Drug Administration. The DV for iron is 18 mg for adults 19 to 50 years old, 27 mg for pregnant women, and 8 mg for adults ≥ 51 years old. Because iron from plants (nonheme iron) is less efficiently absorbed than that from animal sources (heme iron), the recommended DV for iron in a strict vegetarian diet is approximately 1.8 times higher than that for a nonvegetarian diet. Foods providing 20% or more of the DV are considered to be high sources of a nutrient. For more information on the iron content of specific foods, search the USDA food composition database: <http://www.nal.usda.gov/fnic/foodcomp/search/>.

Etiologies of iron deficiency anemia

IDA occurs when iron supply is insufficient to meet the iron requirement of developing RBCs. This occurs secondary to blood loss, increased iron requirements, or inadequate iron supply (Table 6-4). A diagnosis of IDA or iron deficiency alone (without anemia) requires prompt investigation to determine the underlying cause as it may represent the initial presentation of a number of serious diseases.

Table 6-4 Causes of iron deficiency

Blood loss
Menstruation, especially abnormal uterine bleeding or heavy menstrual bleeding
Gastrointestinal (GI) disorders (esophageal varices, hemorrhoids, peptic ulcer disease, malignancy)
Hookworm or other parasitic infections
Rare causes: pulmonary (hemoptysis, pulmonary hemosiderosis), urologic, or nasal disorders
Repeated blood donations without iron replacement, clinical blood draws or factitious blood removal
Dialysis, other intravascular hemolysis with hemoglobinuria (eg, paroxysmal nocturnal hemoglobinuria, prosthetic heart valve)
Increased iron requirements
Rapid growth during infancy, young childhood, and adolescence
Therapy with erythropoiesis-stimulating agents (ESAs)
Pregnancy and lactation
Inadequate iron supply
Poor dietary intake (common in infants and young children; generally not an independent cause in adults)
Malabsorption, duodenum and upper jejunum diseases (celiac disease, gastric bypass surgery, inflammatory bowel disease)
Achlorhydria, autoimmune atrophic gastritis/ <i>Helicobacter pylori</i> colonization
Congenital disorders of iron transport (iron-refractory iron deficiency anemia, hereditary hypotransferrinemia, divalent metal transporter 1 disease)

In young children, IDA is most commonly due to insufficient dietary iron. Those at particular risk are infants primarily breastfed without sufficient iron supplementation beyond 6 months of age and children with excessive cow milk intake (24 ounces per day or greater). Several factors may act synergistically to cause IDA: (i) low iron content in both breast milk and cow milk, (ii) inhibition of nonheme iron absorption by calcium and milk proteins, (iii) potential for occult intestinal blood loss with cow milk protein enteropathy, and (iv) less consumption of other iron-rich foods by those children with excessive milk intake. In adolescent and adult premenopausal women, menstrual blood loss is the most common cause of iron deficiency. Women are at increased risk for IDA during pregnancy, and this is discussed further under “Anemia associated with pregnancy.”

In lower-income countries, hookworm infection resulting in chronic intestinal blood loss is the most common cause of iron deficiency. In higher-income countries, non-parasitic GI blood loss is the most common cause of iron

deficiency in adult males and postmenopausal females. Among those with IDA, evaluation of the GI tract employing endoscopic and radiographic methods identifies a causative lesion in ~60% of cases.

Several additional common GI etiologies of iron deficiency are worth noting. Approximately 5% of patients with IDA referred for hematologic evaluation have subclinical celiac disease, and this number appears to be higher in those patients who are unresponsive to oral iron therapy. In patients with celiac disease, abnormal iron absorption secondary to villous atrophy of the intestinal mucosa and presence of concomitant inflammation likely both contribute to the anemia. It is unclear whether intestinal blood loss also contributes. Although folate and cobalamin deficiency are known complications of celiac disease, IDA is the most common associated nutritional deficiency.

Accumulating evidence supports a significant role of *Helicobacter pylori* infection in the pathogenesis of IDA. A number of proposed mechanisms include occult GI bleeding, competition for dietary iron by the bacteria, and impaired absorption due to the effect of *H. pylori* on digestive fluid composition. Eradication of *H. pylori* colonization, which can coexist and may share a common pathophysiologic mechanism with autoimmune atrophic gastritis in infected individuals with refractory IDA, has been shown to result in an appropriate response to oral iron therapy and normalization of hemoglobin levels. Autoimmune atrophic gastritis (defined as hypergastrinemia and strongly positive antiparietal cell antibodies) is another common cause of IDA.

Iron-refractory iron deficiency anemia is an extremely rare hereditary disease caused by mutations in TMPRSS6, a transmembrane serine protease and characterized by a congenital hypochromic, microcytic anemia, and low serum transferrin saturation. TMPRSS6 mutations result in inappropriately elevated hepcidin levels, resulting in patients being refractory to oral iron and only partially responsive to parenteral iron. Although a congenital disease, we mention it here because genetic variants in TMPRSS6 can determine hemoglobin levels, MCV measurements, and iron status; and may modify response to oral iron therapy in iron-deficient patients.

Stages of iron deficiency and clinical manifestations

The manifestations of iron deficiency occur in several stages (Table 6-5), which are defined by the degree of iron depletion. Initially, iron stores in the bone marrow, liver, and spleen are depleted, which is reflected in decreased serum ferritin. As iron stores become exhausted, TIBC begins to rise and serum transferrin saturation falls. As erythropoiesis becomes iron restricted, cells become microcytic. Anemia is the final manifestation of iron deficiency.

Iron-deficient individuals may be asymptomatic or have nonspecific symptoms of anemia. Pagophagia (craving for ice) and other forms of pica (cravings for nonfood substances) are symptoms more specific for iron deficiency. Findings on physical examination become more pronounced as the iron deficiency worsens and include pallor, stomatitis, glossitis, koilonychia of the nails, and other signs resulting from the effects of iron deficiency on rapidly dividing cells, including the development of red cell hypoplasia. Plummer-Vinson syndrome describes the clinical triad of dysphagia (due to esophageal webs), glossitis, and IDA. Several studies have examined the relationship between iron deficiency and hair loss, primarily in women, with a focus on nonscarring hair loss. However, data have been inconsistent in demonstrating a definitive association.

Diagnosis and treatment

In classic IDA, a patient presents with a clinical history consistent with or concerning for blood loss along with a complete blood count (CBC) demonstrating a microcytic and hypochromic (pale) anemia. An elevated platelet count may also be present. Iron studies reveal a low serum ferritin, serum iron, and transferrin saturation, and elevated transferrin (or TIBC). The peripheral blood smear confirms the microcytosis and hypochromasia and may show increased anisopoikilocytosis (reflected in an increased red blood cell distribution width [RDW]) and bizarrely shaped erythrocytes, including characteristic cigar-shaped or pencil-shaped cells (Figure 6-2). Target cells may be seen and reflect the high area-to-volume ratio of iron-deficient red cells. Table 6-6 compares laboratory assessments found in IDA and anemia of chronic inflammation.

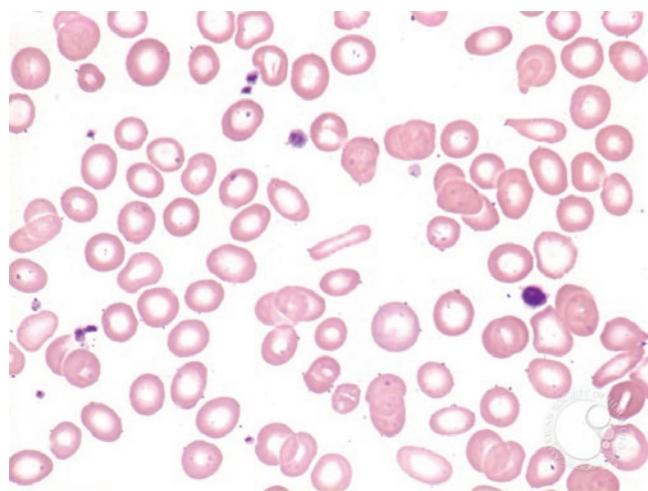
Unfortunately, IDA rarely presents classically and routine iron studies have limitations that complicate the diagnostic algorithm. Serum ferritin is a stable glycoprotein that accurately reflects bone marrow iron stores in the absence of inflammation. In healthy individuals, serum ferritin is directly proportional to iron stores: 1 µg/L serum ferritin corresponds to approximately 8 to 10 mg of tissue iron stores and is an excellent outpatient screen for iron deficiency. In women of reproductive age, a serum ferritin of <10 µg/L is diagnostic of iron deficiency (defined as no stainable bone marrow iron stores) with a reported specificity and sensitivity of approximately 98% and 75%, respectively. A higher serum ferritin cutoff for assessing iron deficiency may be appropriate in some populations. In an anemic patient without inflammation, a serum ferritin of <30 µg/L is 92% sensitive and 98% specific in diagnosing IDA. Ferritin is an acute phase reactant, and its plasma level is increased in liver

Table 6-5 Laboratory findings in progression from normal iron status to iron deficiency anemia

	Normal	Iron depletion	Iron-restricted erythropoiesis	Iron deficiency anemia
Hemoglobin (g/dL)	Normal	Normal	Normal	Decreased
MCV (fL)	Normal	Normal	Slight microcytosis	Microcytic
Serum ferritin ($\mu\text{g}/\text{L}$)*	~ 40 –200	~ 20	~ 10	<10
Iron ($\mu\text{g}/\text{dL}$)	~ 60 –150	$\sim <40$	$\sim <20$	$\sim <10$
TIBC ($\mu\text{g}/\text{dL}$)	Normal	Normal	Normal to mildly increased	Increased
Transferrin saturation (%)	20–50	30	<15	<15
Erythrocyte ZnPP (ng/mL)	~ 30 –70	~ 30 –70	~ 100	~ 100 –200
Marrow sideroblasts	Present	Present	Absent	Absent

ZnPP, zinc protoporphyrin.

*These values represent pure iron deficiency uncomplicated by inflammatory diseases.

**Figure 6-2** Iron deficiency anemia. There are a variety of red blood cell sizes and shapes. Included among these are hypochromic erythrocytes, microcytes, ovalocytes, and “pencil” cells. Source: ASH Image Bank/Peter Maslak.**Table 6-6** Iron studies in iron deficiency anemia vs anemia of chronic inflammation

	Iron deficiency anemia	Anemia of chronic inflammation
Serum ferritin ($\mu\text{g}/\text{L}$)	Decreased	Normal or increased
Iron ($\mu\text{g}/\text{dL}$)	Normal or decreased	Normal or decreased
TIBC; transferrin ($\mu\text{g}/\text{dL}$)	Increased	Normal or decreased
Transferrin saturation (%)	Decreased ($<10\%$ to 15%)	Normal or decreased
MCV (fL)	Decreased	Normal or decreased
RDW	Increased	Normal
sTfR/ \log_{10} ferritin ratio	>2	<1
Hepcidin	Suppressed	Increased

disease, infection, inflammation, and malignancy. Therefore, in patients with chronic inflammatory conditions, evaluation for iron deficiency should include both serum ferritin and transferrin saturation. Serum ferritin of $<100 \mu\text{g}/\text{L}$ and transferrin saturation of $<20\%$ are consistent with iron deficiency. Despite its limitations, low serum ferritin is always consistent with iron deficiency. A serum ferritin of $<30 \mu\text{g}/\text{L}$ is useful in diagnosing iron deficiency in pregnant women (sensitivity of $\sim 90\%$ and specificity of $\sim 85\%$), who often have an elevated serum transferrin in the absence of iron deficiency.

Serum iron and TIBC are unreliable indicators of iron availability to the tissues because of wide fluctuations in

levels resulting from recent ingestion of dietary or medicinal iron, diurnal rhythm, and other factors. Transferrin is affected by nutritional status and transferrin saturation is a calculated measure of serum iron and transferrin.

A number of additional studies can support a diagnosis of IDA when serum ferritin is equivocal. Increased RDW is sensitive for diagnosing IDA, but lacks specificity. A trend of decreasing MCV and increasing RDW over time can be instructive. Erythrocyte zinc protoporphyrin (ZnPP) levels are increased in iron deficiency as a result of zinc, rather than iron, being incorporated into the protoporphyrin ring when iron is unavailable. ZnPP has a high sensitivity for detecting iron deficiency but is

also increased in lead poisoning, anemia of chronic inflammation, and some hemoglobinopathies. The reticulocyte hemoglobin content or equivalent (CHr or Ret-He) is decreased in IDA and is the first peripheral blood marker of iron-deficient erythropoiesis. This test is limited, however, as patients with thalassemia trait also have decreased values, and it requires a specialized analyzer not available in most laboratories. Serum or soluble transferrin receptor (sTfR1) is a circulating protein derived from cleavage of the membrane transferrin receptor on erythroid precursor cells within the marrow. Its level is directly proportional to a person's erythropoietic rate and inversely proportional to tissue iron availability. Iron-deficient patients generally have increased sTfR levels. The incorporation of sTfR1 into the sTfR1-ferritin index ($s\text{TfR}/\log_{10} \text{ ferritin}$) has shown more promise in distinguishing IDA from anemia of chronic inflammation than sTfR1 alone. In patients with iron deficiency, the sTfR-ferritin index is elevated (>2) due to increased erythropoietic drive and low iron stores. In contrast, patients with anemia of chronic disease (AOCD) without concomitant iron deficiency are likely to have an sTfR-ferritin index <1 .

Serum hepcidin, the primary regulator of iron homeostasis, is suppressed in iron deficiency and elevated in persons with anemia of inflammation. The utility of measuring serum hepcidin in the workup of iron deficiency and other disorders of iron homeostasis has not fully been explored, though differentiation between classic IDA and combined IDA and anemia of inflammation is one potential benefit. Recent research assessing serum hepcidin levels in iron-deficient women receiving radiolabeled iron supplementation has demonstrated correspondence between a rise in hepcidin levels post oral iron-dosing and amount of subsequent fractional iron absorption. Therefore, serum hepcidin may benefit in assessing individuals' responsiveness to oral iron therapy. While a clinically validated assay became available in 2017, further investigation of its usefulness in widespread clinical practice is warranted.

Evaluation of the bone marrow for stainable iron was previously considered the gold standard for the diagnosis of iron deficiency. High interobserver variability, expense, and invasiveness of the test limit its clinical utility. This procedure is only indicated in atypical patients in whom there is concern for an underlying malignant or infiltrative process.

Once iron deficiency or IDA is confirmed, evaluation for the underlying etiology (Table 6-4) should be initiated. The diagnostic workup should focus on the likely pathologies based on the clinical history for each specific patient. In premenopausal women, menstrual history including abnormal uterine bleeding or heavy menstrual bleeding should be thoroughly assessed. If menstrual blood loss is

significant and appears to be the primary source of IDA, a trial of iron therapy with close follow-up is reasonable before proceeding to GI studies.

In all male and postmenopausal female patients with confirmed IDA in whom GI blood loss is the most common etiology, upper and lower GI endoscopies should be pursued. Capsule endoscopy to evaluate the small bowel, repeat endoscopic exams, or other diagnostic modalities at the discretion of a gastroenterologist may be required to diagnose obscure GI bleeding (persistent or recurrent bleeding from the GI tract after negative esophagogastroduodenoscopy and colonoscopy). If such evaluations are negative for occult GI blood loss requiring intervention, close follow-up with iron replacement may be a rational approach in some patients.

The definition of *refractory IDA* is not standardized but could be considered in a patient who fails to achieve a 1-g/dL increase in hemoglobin after 4 weeks of at least 100 mg of elemental iron therapy per day. For patients in whom IDA remains unexplained or refractory despite standard diagnostic workup, some experts advocate serological or biochemical screening for celiac disease with antiendomysial or antitransglutaminase IgA antibodies and atrophic gastritis with gastrin and anti-parietal cell antibody testing. Cases of suspected celiac disease should be confirmed by duodenal biopsy. *H. pylori* can be assessed with IgG antibodies or fecal antigen, followed by confirmatory testing with a urea breath test. In patients with iron-refractory or IDA of unknown origin with confirmed *H. pylori* infection, eradication of the infection with standard therapy is reported to be curative and thus should be considered.

An iron absorption test may be useful in evaluating some patients with iron deficiency or IDA. This simple and minimally invasive test distinguishes an intestinal iron absorption defect from other causes of iron deficiency. Ideally, a patient fasts for ~8 hours, and serum iron is measured at baseline and at 90 minutes after administration of ferrous sulfate (65 to 100 mg elemental iron). In a patient with IDA with normal intestinal iron absorption, the serum iron level is expected to increase by at least 100 µg/dL (minimum 50 µg/dL) 90 minutes after the oral iron challenge. The test, however, can be difficult to interpret, particularly in nonfasting patients.

The treatment of iron deficiency or IDA includes addressing the underlying cause of iron deficiency and replacing the iron deficit. Upfront, it is useful to calculate the patient's approximate iron deficit quantitatively. This includes the amount of iron required to normalize the hemoglobin plus the amount of iron required to replete iron stores [the Ganzoni equation: total iron deficit = weight {kg} × (target Hb – actual Hb) {g/L} × 2.4 + iron stores {mg}]. This quantity should be evaluated in the context of

intestinal iron absorption when considering the likelihood of replacing the deficit by oral administration, or to define the amount of parenteral iron to administer.

Oral iron supplementation is the preferred replacement route in most uncomplicated cases of iron deficiency. Iron salts are the most commonly prescribed treatment for iron deficiency. Ferrous sulfate is available in 325 mg (65 mg elemental iron) tablets and ferrous gluconate in 320 mg (32 mg elemental iron) tablets. Ferrous sulfate elixir (a liquid formulation) is available for infants and young children. In addition to salts, formulations of iron polysaccharide complex and carbonyl iron are available and may be better tolerated. However, most data demonstrate superiority of iron salts due to enhanced absorption compared to these alternative forms. Historically, typical replacement doses of elemental iron in adults ranged from 100 to 200 mg/day and 3 to 6 mg/kg/day in infants and children administered from 1 to 3 times daily. However, recent research has demonstrated in both adults and children that lower doses may be better tolerated, allow for improved adherence, and result in higher fractional iron absorption compared to multiple daily doses. A study of iron-deficient, nonanemic healthy women found that cumulative iron absorption was greater in those receiving alternate-day dosing of oral iron than in those receiving daily dosing. Similar studies are needed for iron-deficient patients with anemia to determine whether the same holds true for that population. However, 65 mg of elemental iron per day in adults and 3 mg of elemental iron per kilogram per day in children, administered once daily, are likely sufficient in the majority of IDA patients.

Nausea, vomiting, epigastric discomfort, and constipation are common dose-dependent side effects of iron salts; approximately 25% of patients cannot tolerate oral iron because of side effects. Patients should be alerted that iron darkens stools. Oral iron salts are absorbed best on an empty stomach but are better tolerated when taken with foods. Ascorbic acid can facilitate iron absorption, but its addition to the replacement regimen is not clearly cost effective and may increase the adverse effects of iron replacement therapy. An alternative approach is to instruct patients to take oral iron supplements with orange juice. Some evidence suggests that even lower doses of oral iron (ie, a single daily dose of 25 mg of elemental iron) remain effective and result in lower rates of adverse effects, though it is unknown whether such a low dose regimen requires longer duration of therapy. Antacids, the tannins found in tea, calcium supplementation, bran, and whole grains can all decrease iron absorption if taken concurrently with oral iron. Treatment with oral iron to replenish iron stores should continue for approximately 3 months after the hemoglobin normalizes.

Oral heme iron polypeptide, derived through the proteolytic digestion of porcine hemoglobin, is another available oral iron formulation. Heme iron (derived from hemoglobin and myoglobin in animal food sources) is more efficiently absorbed and via a different, undefined mechanism, than nonheme iron. Limited data compare oral heme iron polypeptide to other oral iron formulations; therefore, its true efficacy is unknown. It is currently more expensive than oral iron salts.

Parenteral iron should be given intravenously and is indicated when there is an absolute nonadherence with or intolerance to oral iron therapy, high iron requirements, or proven intestinal malabsorption. Long-term ramifications of IV iron therapy remain unstudied. Multiple parenteral iron preparations are now available in the United States. High-molecular-weight iron dextran is complicated by a low but significant risk of anaphylaxis (11.3 per million), and thus should no longer be used. Low-molecular-weight iron dextran is considerably safer than its high-molecular-weight counterpart but carries a black box warning and requires a test dose prior to full dose infusion. The advantages of low-molecular-weight iron dextran include its low cost and the ability to give replacement doses of iron in a single or “total-dose” infusion. Iron sucrose and ferric gluconate both have very low incidence of anaphylaxis, and their administration does not require a test dose. Side effects of iron sucrose and ferric gluconate include mild arthralgia and myalgia. The principal disadvantage is the inability to give a total replacement dose in a single infusion, with a typical limitation of 200 to 300 mg per infusion. GI and vasoactive reactions occur at doses greater than 200 to 400 mg. Newer iron preparations have been developed to enable more rapid high-dose bolus injections. Ferumoxytol, licensed for use in adult patients with chronic kidney disease and IDA, enables a bolus injection of 510 mg to be administered in 17 seconds. It, too, carries a black box warning due to low but serious risk of severe and potentially fatal allergic reactions. Ferric carboxymaltose was licensed in the United States in 2014 for patients with IDA who are intolerant of oral iron therapy. It can be administered at a maximum single-infusion dose of 750 mg over 15 minutes for patients weighing >50 kg.

IDA patients receiving supplemental iron generally demonstrate reticulocytosis within 7 to 10 days of initiating treatment. Hemoglobin response generally occurs within 2 weeks but may take longer to fully correct, and serum ferritin should correct once additional iron (beyond that to correct the hemoglobin) accumulates to replenish body stores. Failure to respond to oral iron should prompt consideration of patient nonadherence, inadequate replacement dosing, poor iron absorption, ongoing blood loss, or appropriateness of the diagnosis.

KEY POINTS

- Iron deficiency is the most common cause of anemia worldwide, and its diagnosis requires an evaluation for the underlying etiology.
- Young children most commonly have nutritional IDA due to insufficient dietary iron, while adolescent and adult premenopausal women are at risk for IDA due to chronic menstrual blood loss.
- In adult men and postmenopausal women, GI blood loss is the most common cause of IDA.
- IDA is the most common nutritional deficiency associated with celiac disease.
- Classic iron deficiency is characterized by a hypochromic, microcytic anemia, elevated RDW, and low corrected reticulocyte count.
- A ferritin of <10 µg/L in any individual is diagnostic of iron deficiency.
- Oral iron supplementation is the preferred replacement route in most uncomplicated cases of iron deficiency.
- Failure to respond to oral iron should prompt consideration of ongoing blood loss, inadequate replacement dosing, or poor absorption due to underlying GI pathology (eg, celiac disease, *H. pylori* infection, or atrophic gastritis).

Normocytic anemias

Anemia of chronic inflammation (anemia of chronic disease)

CLINICAL CASE

A 44-year-old woman is referred for evaluation of a hypoproliferative normocytic anemia with a hemoglobin of 8 g/dL. Her past medical history is significant for a mitral valve replacement 1 year earlier. Recently, she has developed low-grade fevers, malaise, and generalized fatigue. Her examination is remarkable for a temperature of 38.5°C and a 2/6 systolic ejection murmur over the mitral valve. Laboratory evaluation reveals that serum ferritin is 55 ng/mL, transferrin saturation is 12%, and an elevated erythrocyte sedimentation rate (ESR) at 92 mm/h. Blood cultures subsequently return positive for α -hemolytic streptococci. Transesophageal echocardiogram confirms subacute bacterial endocarditis of the prosthetic mitral valve. The patient is treated with penicillin and gentamicin. Four weeks later, the hemoglobin increases to 11 g/dL.

Overview

Anemia is common in patients with chronic inflammatory conditions such as malignancy, autoimmune disease, chronic infection, and chronic kidney disease. The resulting anemia is termed the *anemia of chronic inflammation* or the *anemia of chronic disease*. It is now recognized that patients with conditions not traditionally thought to be inflammatory, such as trauma, postsurgical, and periods of prolonged critical illness, may also develop AOCD. AOCD is reflective of underlying disease activity and evaluation for an underlying disorder is warranted when diagnosing AOCD as the cause of anemia. Patients with AOCD typically have hemoglobin levels in the range of 7 to 11 g/dL. AOCD is characterized as a normochromic, normocytic anemia with a low corrected reticulocyte count. Over time, however, the anemia may become more severe with microcytic and hypochromic indices. Although laboratory values overlap and may not assist in differentiation, iron studies are often used to distinguish AOCD from IDA. In AOCD, serum iron and iron-binding capacity are typically low to normal, and ferritin is normal or elevated (Table 6-6). In many but not all conditions, an elevated ESR or C-reactive protein supports the diagnosis of AOCD.

Multiple processes are involved in the pathogenesis of AOCD. Cytokines, such as tumor necrosis factor alpha, interleukin 1, interleukin 6, and interferons play a central role. These cytokines cause a reduction in the proliferation of erythroid precursors in response to EPO, a decrease in the EPO production relative to the degree of anemia, and a moderate decrease in RBC survival. The hallmark of AOCD is an alteration in iron metabolism. Inflammatory cytokines, especially IL-6, increase hepatic synthesis of hepcidin, the key regulator of cellular iron homeostasis. As previously mentioned, hepcidin acts by binding the iron export protein, ferroportin, leading to its degradation and thereby inhibiting intestinal iron absorption and macrophage iron recycling. This results in iron-restricted erythropoiesis and is reflected in low plasma iron and transferrin saturation levels.

In infants and children, anemia due to inflammation does not require the presence of an underlying chronic inflammatory disorder. Minor acute bacterial or viral infections, when recurrent, can cause a mild normocytic anemia with blunted reticulocyte response within a few weeks. The pathophysiology likely mirrors AOCD. This form of anemia of inflammation is self-limited and resolves when the infant or child's infection resolves.

In most patients, AOCD is mild and improves with treatment of the underlying disorder. Patients may have, however, concomitant iron deficiency or functional iron deficiency. If treatment becomes necessary, erythropoiesis-stimulating agents (ESAs) have been shown to be beneficial

in some patients in addition to supplemental iron, particularly parenteral iron. Future treatment options for AOCD may involve decreasing hepcidin. Most data on ESAs and supplemental iron to correct an acquired underproduction anemia come from patients with chronic kidney disease. AOCD is one mechanism contributing to this form of anemia (discussed further in the next section).

KEY POINTS

- AOCD is the most common cause of anemia in patients with underlying inflammatory diseases.
- AOCD is characterized by a normocytic, or microcytic anemia and low corrected reticulocyte count. Iron studies typically demonstrate decreased serum iron, normal or decreased transferrin (or TIBC) and decreased transferrin saturation, along with a normal or increased serum ferritin.
- The pathophysiology of AOCD is multifactorial, but the sequestration of iron secondary to elevation in serum hepcidin plays a central role.
- Primary treatment should be directed at the underlying medical condition.

Anemia associated with chronic kidney disease

CLINICAL CASE

A 71-year-old woman presents to her primary care physician with increasing dyspnea on exertion. She is found to have a hypoproliferative, normocytic anemia (hemoglobin 9.5 g/dL), and a creatinine of 2.2 mg/dL. Iron studies were normal. She was started on an ESA along with an oral iron supplement. Within 4 weeks, she had good clinical response; however, 2 months later she returns with recurrent exertional dyspnea. Laboratory values reveal a hemoglobin of 9.7 g/dL and an MCV of 77 fL. Iron studies are consistent with IDA. Intravenous iron dextran is administered with good clinical response.

Overview

Anemia of chronic kidney disease (CKD) is primarily due to underproduction of EPO. This is a result of a decrease in the number of renal cortical cells available to produce the hormone, as well as the accumulation of uremic toxins. Iron-restricted erythropoiesis due to AOCD contributes to anemia of CKD as well. Excessive hepcidin in CKD patients may be due in part to its reduced renal clearance and/or increased inflammatory-mediated expression. Hepcidin levels in CKD are further influenced by iron and ESA administration. As in other patients with AOCD, elevation in serum hepcidin impairs both dietary iron

absorption and iron release from body stores. AOCD in patients with CKD may account for ESA resistance observed in some patients. Additional factors contribute to the anemia of CKD, including impaired RBC deformability and membrane permeability secondary to uremia and secondary hyperparathyroidism. Hemodialysis may result in RBC fragmentation and increased blood loss as well as exposure to RBC toxins. Increased iron demand and utilization related to ESA therapy also contributes to anemia of CKD.

Anemia of CKD is typically normochromic and normocytic with a low reticulocyte count, unless complicated by iron deficiency or other vitamin deficiencies. Peripheral smear is often normal, but in rare patients with severe kidney failure, echinocytes can be seen (characterized by irregular broad-based short blunt projections of the RBC membrane).

The bone marrow is normocellular, but a hypocellular marrow with relative erythroid hypoplasia and marrow fibrosis (osteitis fibrosa) related to secondary hyperparathyroidism has been described. Iron studies may be normal or may show low serum iron levels accompanied by low serum transferrin and elevated serum ferritin, as seen in AOCD.

It is well established that recombinant human EPO and other ESAs improve the anemia associated with CKD. However, use of ESAs in CKD patients to target normal or near-normal hemoglobin values is associated with increased risk of adverse cardiovascular events and mortality. Conversely, only modest to minimal improvement in patient-reported outcomes such as fatigue have been demonstrated with ESAs' targeting of lower target hemoglobin levels. Randomized control studies are needed to define both the optimal hemoglobin concentration for initiation of ESA therapy and a therapeutic target to achieve improved clinical and patient-reported outcomes, as well as the best use of intravenous iron to reduce total ESA dosing requirements. The National Kidney Foundation Kidney Disease Outcomes Quality Initiative and Kidney Disease: Improving Global Outcomes provide nephrologists with guidelines on the management of anemia associated with CKD based on available evidence and expert opinion.

KEY POINTS

- Anemia associated with CKD is primarily due to an EPO deficiency. However, multiple pathophysiologies likely contribute, including AOCD.
- ESAs are effective treatment of anemia associated with CKD.
- ESAs and IV iron treatment of anemia associated with CKD require careful consideration of the potential benefits and harms to the individual patient.

Macrocytic anemias

Megaloblastic anemias

Megaloblastic anemias result from impaired DNA synthesis in hematopoietic cells and are characterized by macrocytosis with marked variation in the size and shape of red blood cells (often macro-ovalocytes), a low corrected reticulocyte count, hypersegmented neutrophils, and occasionally pancytopenia. Megaloblastic changes in the marrow result from the dyssynchrony between nuclear and cytoplasmic maturation and include: hypercellular marrow with an erythroid predominance (reversed myeloid:erythroid ratio), presence of giant pronormoblasts, and giant metamyelocytes. An imbalance of iron quantity endocytosed by marrow erythroblasts vs that incorporated into circulating erythrocytes, reflects ineffective erythropoiesis and implies cell death within the marrow during erythroid differentiation. Peripherally, these changes are reflected by elevated lactic dehydrogenase, elevated unconjugated bilirubin, and low haptoglobin and the occasional appearance of red cell fragments on peripheral smear.

Megaloblastic anemias and MDS share a number of clinical findings, and MDS should be considered in the differential diagnosis during initial evaluation. Hypercellular marrow with giant pronormoblasts, as seen in megaloblastic anemias, may also lead to an incorrect diagnosis of acute leukemia. Cobalamin and folate deficiencies are the most common causes of megaloblastic anemias.

Vitamin B₁₂ deficiency

CLINICAL CASE

A 75-year-old man is referred by his urologist for investigation of anemia. He has a diagnosis of transitional cell carcinoma of the bladder for which he has been treated with transurethral resection and an intravesical bacillus Calmette-Guérin vaccine. He has type 2 diabetes treated with metformin. On examination, he is pale, has mild peripheral edema, and minimal loss of position and vibratory senses in the feet bilaterally. Laboratory evaluation reveals a hemoglobin of 7.1 g/dL, MCV of 109 fL, neutrophil count of 960/ μ L, and platelet count of 35,000/ μ L. Serum cobalamin level is 72 pg/mL, serum folate is normal, and RBC folate is mildly depressed. He is started on daily parenteral cobalamin replacement, with symptomatic improvement and brisk reticulocytosis noted within 1 week.

Background

Vitamin B₁₂ (cobalamin) functions as an essential coenzyme for cytoplasmic methionine synthase and methylmalonyl-CoA mutase. Cytoplasmic methionine synthase catalyzes

methylation of homocysteine to methionine, which is linked to folate metabolism, as the methyl group transferred to homocysteine is provided by the conversion of 5-methyl tetrahydrofolate to tetrahydrofolate. Tetrahydrofolate is essential for purine and pyrimidine synthesis. Methylmalonyl-CoA mutase catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA in the mitochondria, and succinyl-CoA then enters the Krebs cycle.

Humans are completely dependent on dietary (predominantly animal) sources of cobalamin. In the stomach, released cobalamin is bound to the protein haptocorrin, present in saliva and gastric fluids, which protects the vitamin from degradation within the acidic stomach environment. In the duodenum, pancreatic enzymes degrade haptocorrin and cobalamin subsequently binds to intrinsic factor, which is synthesized and secreted by the parietal cells of the stomach. Intrinsic factor-bound cobalamin is endocytosed by the receptor complex cubam in the terminal ileum. Inside the ileal enterocyte, intrinsic factor is degraded and released cobalamin exits the basolateral cell surface via a transporter. In the plasma, cobalamin binds to transcobalamin II for delivery to tissues.

Cobalamin deficiency results most commonly from abnormal intestinal absorption; or rarely, from insufficient dietary intake or defects in bodily transport. Select causes of cobalamin deficiency are listed in Table 6-7. Due to efficient enterohepatic circulation and renal reuptake, cobalamin is retained in the body for long periods, and dietary cobalamin deficiency therefore develops slowly over a period of years.

The most common cause of symptomatic cobalamin deficiency is pernicious anemia (PA). PA is an autoimmune disorder in which antibodies to gastric parietal cells cause gastritis and mucosal atrophy of the body and fundus of the stomach. Such atrophy reduces the number of parietal cells that produce intrinsic factor, required for vitamin B₁₂ absorption; which, in turn, is required for erythropoiesis and myelin synthesis. PA is frequently associated with other autoimmune disorders (eg, type 1 diabetes, autoimmune thyroiditis, primary hyperparathyroidism, and vitiligo). Diagnosis includes demonstration of a megaloblastic anemia, low serum vitamin B₁₂ level, and the presence of antibodies to intrinsic factor, which are less sensitive but more specific than parietal cell antibodies. The gastric enzyme H⁺/K⁺ATPase is the target antigen recognized by parietal cell antibodies but may be positive in persons with other autoimmune diseases, as well as healthy individuals, and is therefore less helpful. Stomach biopsy or serum biomarkers consistent with chronic atrophic gastritis are not required for the clinical diagnosis of PA.

Some data suggest long-standing *H. pylori* infection in the pathogenesis of PA and atrophic body gastritis. One hypothesis suggests that over time the infection is replaced

Table 6-7 Select causes of vitamin B₁₂ deficiency

Impaired absorption
Deficiency of intrinsic factor or IF-bound vitamin B ₁₂ uptake; congenital intrinsic factor deficiency
Pernicious anemia or other gastric atrophy (<i>Helicobacter pylori</i> or autoimmune gastritis)
Gastric bypass surgery
Decreased ileal absorption of vitamin B ₁₂ (Imerslund-Gräsbeck syndrome)
Hypochlorhydria (impairs release of B ₁₂ from dietary proteins)
Age
Medications (proton-pump inhibitors, H ₂ antagonists, metformin [mechanism unknown], nitrous oxide abuse)
Inadequate pancreatic protease (vitamin B ₁₂ remains sequestered by haptocorrin)
Intestinal competition for host vitamin B ₁₂ (tapeworm <i>Diphyllobothrium latum</i>)
Ileal resection, bypass or dysfunction (Crohn disease, celiac disease, intestinal lymphoma, bacterial overgrowth from blind loop syndrome)
Insufficient dietary intake
Strict vegans, some vegetarians, breastfed infants of vitamin B ₁₂ -deficient mothers
Defects in bodily transport
Congenital disorders of vitamin B ₁₂ transport (defects in cubam, transcobalamin, others)

IF, intrinsic factor.

by an autoimmune process mediated by autoreactive gastric T-cells, which recognize H⁺/K⁺-ATPase and *H. pylori* antigens. These autoreactive cells cause irreversible mucosal damage. It is unclear whether PA should be included among the long-term consequences of *H. pylori* gastritis, and thus *H. pylori* testing in the evaluation of patients with PA remains controversial.

PA patients are at risk for development of gastric adenocarcinoma and carcinoid tumors. Data are insufficient to support routine subsequent endoscopic surveillance of these patients, however, and follow-up should be individualized to the patient.

Nitrous oxide is associated with an acute megaloblastic anemia secondary to impaired cobalamin metabolism. Abuse of this compound has been associated with psychosis and other neurologic defects.

Diagnosis

Cobalamin deficiency can present insidiously with unexplained anemia, neuropsychiatric symptoms, or GI manifestations, including swollen or sore tongue (glossitis), anorexia, and diarrhea. Neurologic symptoms include paresthesia, unsteady gait or clumsiness, and motor weakness

progressing to paralysis. Psychiatric symptoms include mania, paranoia, and irritability. Within the nervous system, cobalamin deficiency leads to defective myelin synthesis resulting in central and peripheral nervous system dysfunction. In the spinal cord, subacute combined degeneration occurs, affecting its posterior and lateral columns, which presents clinically as loss of vibratory sense and proprioception. Magnetic resonance imaging (MRI) shows symmetrical increased T2 signal intensity in the posterior or posterolateral columns, commonly confined to the cervical and thoracic spinal cord. Brain involvement in cobalamin deficiency on MRI has also been reported.

Early recognition of these signs and symptoms is critical to avoid irreversible neurologic dysfunction. While both folate and cobalamin deficiencies result in megaloblastic anemia, only cobalamin deficiency results in neuropsychiatric symptoms. Therefore, cobalamin levels always should be measured before initiation of folate in patients at risk for concomitant cobalamin deficiency. Folate replacement alone may improve anemia in patients with cobalamin deficiency, thereby masking the underlying cobalamin deficiency, which allows for progression of neurologic deficits. While hematologic changes are typically present early, some patients may present with neurologic involvement in the absence of accompanying anemia. It is unknown why some patients develop one set of symptoms over the other. However, macrocytic anemia cannot be used as the sole criterion for pursuing the diagnosis. Accordingly, any patient with unexplained neuropathy should be assessed for cobalamin deficiency.

Cobalamin deficiency is a rare and treatable cause of failure to thrive and delayed development in infants. Its long-term developmental consequences remain unknown. In developed countries, deficiency can occur in infants exclusively breastfed by mothers who are themselves deficient in cobalamin (eg, unrecognized PA, strict vegetarian or vegan diet), causing low cobalamin body stores in the infant at birth and inadequate amounts of cobalamin in the breast milk. Signs and symptoms often present between the ages of 4 and 12 months and include failure to thrive, lethargy, hypotonia, and arrested or regressed developmental skills. It can rarely cause seizures or brain atrophy on imaging. Infants often demonstrate abnormal movements, including tremor, myoclonus, and choreoathetoid movements.

Rare cases of cobalamin deficiency due to a congenital defect in intrinsic factor secretion from parietal cells (ie, congenital PA) present around 18 to 36 months of age, after the depletion of fetal liver stores. Acquired PA may present in children as well. The Imerslund-Gräsbeck syndrome is a rare congenital defect in cobalamin absorption resulting from mutations in the cubam receptor complex. In some cases, this autosomal recessive disorder also causes

proteinuria, which is related to cubam's function in the renal reabsorption of some filtered proteins. Transcobalamin II deficiency is inherited as an autosomal recessive trait that presents in early infancy with severe megaloblastic anemia despite the presence of normal intrinsic factor secretion, cobalamin absorption, and cobalamin levels.

No gold standard test exists for diagnosing cobalamin deficiency because each laboratory test has its disadvantages. A serum cobalamin assay, which quantifies all forms of cobalamin in serum, is the standard initial routine diagnostic test. It is a widely available, inexpensive, and automated method based on intrinsic factor binding of cobalamin and immune chemoluminescence. Unfortunately, the assay lacks sensitivity and specificity and demonstrates highly variable results. Both significant intraindividual variation and large absolute differences in results may be seen on repeat testing. In patients in whom cobalamin deficiency is clinically suspected, a serum cobalamin level <200 pg/mL supports the diagnosis. It is important to note that given the significant diagnostic limitations of serum cobalamin measurements, values above this cutoff do not exclude the diagnosis and hematologists need to carefully consider the clinical scenario of each case. For example, spuriously high cobalamin levels have been reported in patients with PA, which has been attributed to assay interference by high levels of antibodies against intrinsic factor. Adequate cobalamin treatment is the safest approach if the clinical presentation and laboratory studies are confusing. Complete resolution of symptoms with therapy supports the diagnosis of cobalamin deficiency. Once cobalamin deficiency is diagnosed, evaluation for the underlying cause is necessary.

Methylmalonic acid (MMA) and total homocysteine (HCY) are more sensitive indicators of early cobalamin deficiency as serum levels of both MMA and HCY become elevated before cobalamin levels fall below the lower limits of the normal range. Elevations in one or both have been shown to correlate with clinical response to therapy. In patients with equivocal serum cobalamin levels and in whom clinical suspicion persists, metabolite testing with MMA and HCY is reasonable. Testing MMA and HCY levels is reasonable in patients with atypical clinical findings in whom cobalamin deficiency is being considered, and in asymptomatic patients incidentally found to have a low cobalamin level. HCY levels lack specificity and can be elevated in patients with folate deficiency, renal dysfunction, and other settings. There is debate regarding the clinical importance of laboratory tests suggesting cobalamin deficiency in patients without overt cobalamin deficiency symptoms (ie, absence of neurologic and hematologic findings), so-called subclinical cobalamin deficiency. Many patients with subclinical cobalamin deficiency do not pro-

gress to symptomatic cobalamin deficiency. It remains unknown whether these patients have subtle and clinically unrecognized symptoms of cobalamin deficiency. It is debated whether treatment and/or close follow-up is indicated. These discrepancies reflect a lack of uniform diagnostic criteria for subclinical cobalamin deficiency and the limitations in laboratory testing for cobalamin deficiency. Therefore, routine screening of asymptomatic individuals for cobalamin deficiency is not recommended.

Low cobalamin levels alone (without megaloblastic anemia or neurological symptoms) may be seen in association with a variety of conditions, including pregnancy (due to changes in protein binding), folate deficiency, and use of certain drugs (eg, oral contraceptives and metformin). True cobalamin deficiency in these situations can be confirmed by elevations in MMA and HCY levels. Other conditions can cause an elevated level of HCY alone (hypothyroidism, vitamin B₆ deficiency), MMA alone (intestinal overgrowth), or both (renal failure).

Testing for intrinsic factor antibodies alone may be performed in patients with evidence of low cobalamin; additional testing for nonspecific serum gastrin or pepsinogen levels in individual cobalamin-deficient patients (predicted to be elevated and low in deficient patients, respectively) may be indicated as well. The incidence of intrinsic factor antibodies increases to 60% to 80% with increasing disease duration. As cobalamin therapy can cause false-positive results on intrinsic factor antibody testing, assessment should occur at least a week after a cobalamin injection to ensure accurate results. Parietal cell antibodies are present in 80% to 90% of PA patients, especially in the early stages of the disease. Later in the disease course, the incidence of parietal cell antibodies decreases due to the progression of autoimmune gastritis and loss of gastric parietal cell mass. Parietal cell antibodies lack specificity and can also be found in other autoimmune diseases (ie, Hashimoto thyroiditis or type 1 diabetes) or in elderly subjects, at low frequency. Historically, the Schilling test was used to measure cobalamin absorption, but this test is no longer available in most centers.

Treatment

Patients with cobalamin deficiency can be treated with parenteral or oral cobalamin replacement. Parenteral therapy is recommended for patients with significant symptoms. Intramuscular cobalamin is given in doses of 1,000 µg/day (up to 150 µg is retained from each injection by most patients) for 1 week, then 1,000 µg/weekly for 4 weeks, and then 1,000 µg/month or less frequently. Alternative dosing regimens can be used. Excess cobalamin is excreted in the urine, so toxicity due to excessive vitamin replacement does not occur. The observation of intrinsic factor-unrelated

diffusion of ~1.2% of oral cobalamin (any dose) suggests that oral cobalamin may be a safe and effective treatment in some patients, even with low levels of intrinsic factor. The initial oral replacement dose begins at 1,000 to 2,000 µg/day. Patients should be observed carefully to ensure that symptoms of anemia improve. After cobalamin replacement is initiated, some patients become iron deficient due to more efficient iron uptake by developing erythroid cells, which then requires iron replacement as well.

Following cobalamin replacement, the bone marrow shows resolution of megaloblastic changes within hours. Reticulocytes appear in the peripheral blood, typically peaking approximately 1 week after initiating replacement therapy. Neutrophil hypersegmentation may persist for up to 2 weeks. Blood counts and MCV return to normal in 2 to 3 months. Neurologic abnormalities usually improve within 3 months; though in some patients, this may take up to 12 months. In some individuals, the neurological deficits are irreversible.

KEY POINTS

- The most common etiology of cobalamin deficiency is impaired absorption, typically due to pernicious anemia, which results in symptomatic deficiency.
- Both cobalamin and folate deficiencies cause a megaloblastic anemia; however, neuropsychiatric symptoms are seen only in cobalamin (vitamin B₁₂) deficiency.
- Subclinical cobalamin deficiency (defined by elevated MMA and HCY levels with no clinical signs or symptoms) is of uncertain significance.
- Parenteral cobalamin replacement therapy is recommended for patients with any neuropsychiatric symptoms.

Folate deficiency

CLINICAL CASE

A 55-year-old man presents for routine physical examination. He complains of fatigue and shortness of breath. He admits to daily excessive alcohol consumption since he lost his job 6 months ago. Physical examination reveals pallor, glossitis, a flow murmur, and a normal neurological examination. Laboratory evaluation reveals a hemoglobin of 7.1 g/dL, MCV of 130 fL, neutrophil count of 1,000/µL, and platelet count of 55,000/µL. A serum folate level is 1 ng/mL, cobalamin level is 350 pg/mL. He is enrolled in an alcohol treatment program and started on 2 mg of daily oral folic acid replacement with symptomatic improvement and brisk reticulocytosis noted within 2 weeks.

Background

Folate exists in nature as a conjugate with glutamic acid residues. Folate, when reduced to tetrahydrofolate, is involved in 1 carbon metabolism. Thus, it is critical for the synthesis of purines and pyrimidines, and for amino acid metabolism. Though rare, a loss-of-function mutation in the gene encoding a proton-coupled high-affinity folate transport protein (PCFT/HCP1) within the duodenum and jejunum results in a syndrome of hereditary folate malabsorption.

Folate deficiency is rare and significantly less common than cobalamin deficiency. It may result from impaired absorption (ie, sprue, Crohn disease, or celiac disease) or increased utilization (Table 6-8), but the principal cause is decreased dietary intake. Green leafy vegetables, citrus fruits and juices, dried beans, and peas are all natural sources of folate. The implementation of folic acid fortification in grains has drastically reduced the prevalence of folate deficiencies in many countries. The FDA-recommended daily dietary folate equivalent is 400 µg. Folate deficiency due to inadequate dietary intake can develop within a few months because body stores are not extensive. Folate supplementation should be part of routine prenatal care to reduce the risks of neural tube defects in infants, and should also be considered in other patients with increased folate requirements (eg, some forms of chronic hemolysis with high red cell turnover, such as pyruvate kinase deficiency, and some sickle cell disease patients).

Diagnosis and treatment

The hematologic manifestations of folate deficiency are indistinguishable from cobalamin deficiency. However, folate deficiency does not cause subacute combined degeneration. Folate deficiency is strongly implicated in increasing the incidence of fetal neural tube defects. Plasma (or serum) folate undergoes diurnal changes related to recent food intake, which limits the usefulness of the diagnostic assay. If the serum folate is >4 ng/mL, folate deficiency is unlikely. A serum folate concentration of <2 ng/mL is more consistent with folate deficiency. Alternatively, RBC folate levels have less daily variability and more accurately reflect the average folate content of the circulating RBC population. However, RBC folate levels may also be low in persons with cobalamin deficiency. Folate deficiency results in high levels of HCY, but not MMA. Assessment for cobalamin deficiency should always be performed prior to initiation of folate therapy because folic acid can partially reverse the hematologic abnormalities of cobalamin deficiency, while the neurologic symptoms resulting from cobalamin deficiency progress. Treatment with folic acid (1 to 5 mg per day) should be prescribed for 1 to 4 months,

Table 6-8 Select causes of folate deficiency

Insufficient dietary intake
Poor intake of fruits and vegetables or prolonged cooking of these foods
Alcoholism (alcohol increases renal folate excretion and impairs intracellular metabolism)
Impaired absorption
Intestinal dysfunction (Crohn disease, celiac disease)
Congenital abnormality in intestinal folate transporter (mutations in <i>PCFT</i>)
Increased requirements
Increased cellular proliferation
Pregnancy and lactation
Hemolytic anemia (sickle cell anemia, warm autoimmune hemolytic anemia)
Malignancies (associated with a high proliferative rate)
Exfoliative dermatitis
Hemodialysis
Medication affecting folate metabolism or absorption (methotrexate, phenytoin, carbamazepine)

or until complete hematologic recovery occurs. Folate is inexpensive and effective even in persons with malabsorption.

KEY POINTS

- The most common cause of folate deficiency is decreased dietary intake.
- Folate supplementation should be part of routine prenatal care.
- Patients with some forms of chronic hemolytic anemia (eg, pyruvate kinase deficiency) should receive daily folate supplementation.
- HCY is elevated, and MMA is normal in folate deficiency.
- Cobalamin deficiency should be ruled out prior to initiating treatment with folate.

Other causes of megaloblastic anemia

In addition to folate and cobalamin deficiency, there are other rarer causes of megaloblastic anemia. Drugs that affect DNA synthesis are the most likely cause of megaloblastic anemia in the absence of folate and cobalamin deficiency. The most common drugs include 5-fluorouracil (pyrimidine analog), azathioprine (purine analog), and methotrexate (antifolate). Hydroxyurea, zidovudine, and several anticonvulsant medications also likely inhibit DNA synthesis.

Acquired pure red cell aplasia

CLINICAL CASE



A 64-year-old female presents with fatigue and dyspnea on exertion, which has been progressive over the last 2 months. She is not taking any medications and has no significant past medical history. Previous blood counts reportedly have been normal. Physical examination is significant for skin pallor and pale conjunctivae. Laboratory evaluation reveals hemoglobin of 6.4 g/dL, MCV of 99 fL, absolute reticulocyte count of <10,000/ μ L, and corrected reticulocyte count of 0.3%. White blood cell and platelet counts are normal. Bone marrow examination reveals a maturation arrest at the proerythroblast stage. Flow cytometry does not reveal a lymphoproliferative disorder, and cytogenetic evaluation results are normal. Computed tomography (CT) scan of the chest fails to identify a thymoma. Prednisone 1 mg/kg daily is prescribed, and within 2 weeks, a partial response is seen. After 6 weeks, a complete response is seen, and a slow taper of prednisone is begun. The patient relapses after prednisone withdrawal. She is begun on cyclosporine with a gradual but complete response in her blood counts.

Background

Pure red cell aplasia (PRCA) is characterized by a severe normochromic, normocytic or macrocytic anemia with reticulocytopenia, and either an absence of hemoglobin-containing cells (<3% of the nucleated marrow cells) or maturation arrest at the proerythroblast stage (Figure 6-3). If PRCA is secondary to large granular lymphocyte leukemia or another lymphoproliferative disorder, the marrow shows lymphocytic infiltration.

PRCA occurs as either an acquired or congenital (Diamond-Blackfan anemia; see Chapter 15) disorder. Acquired PRCA is further classified as primary or secondary, depending on the absence or presence of an associated disease, infection, or drug (Table 6-9). Alternatively, acquired PRCA can be classified by the pathophysiology of the anemia. Erythropoiesis can fail by 3 distinct mechanisms. In most cases of PRCA, an aberrant immune response leads to suppression of RBC development: erythroid progenitor cells are intrinsically normal but their differentiation is inhibited by a humoral or T-lymphocyte-mediated mechanism. The majority of cases are idiopathic. In about 10% of cases, PRCA results from chronic parvovirus B19 infection, and in rare cases, PRCA is the initial clinical manifestation of an MDS.

Several causes of acquired PRCA are reviewed here. Transient erythroblastopenia of childhood is an acquired PRCA observed in infants and young children. Affected

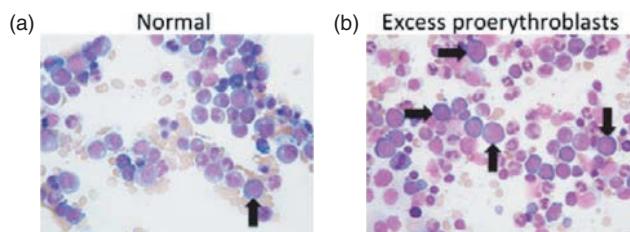


Figure 6-3 Pure red cell aplasia bone marrow aspirate with excess proerythroblasts. Arrows indicate proerythroblasts. Wright-Giemsa-stained marrow aspirates from a normal patient (a) and a pure red cell aplasia patient (b) are shown.

patients are usually between 6 and 36 months of age, otherwise healthy, and present only with the insidious onset of pallor or incidental finding of a normocytic anemia. The degree of anemia is variable but may become severe, and mild neutropenia may also be present. The differential diagnosis includes Diamond-Blackfan anemia (typically MCV is elevated) and parvovirus B19 infection. Although the pathophysiology is not well characterized, most cases appear to be due to an antibody (IgG) directed against erythroblasts. The condition resolves spontaneously within weeks or months with no sequelae. Treatment is supportive, and transfusion should be avoided unless the patient becomes symptomatic.

Parvovirus B19 is a common infection in children, causing erythema infectiosum (ie, fifth disease). More than 75% of adults >50 years old have neutralizing antibodies against this virus. In all infected patients, the virus binds to the blood group P antigen expressed on erythroid progenitors and is cytotoxic to the infected cells. In patients with normal immunity, high-titer parvovirus persists in the blood and marrow for 2 to 3 weeks and is then cleared. Because the normal life span of the RBC is 120 days, infection does not immediately result in a significant decrease in hemoglobin. Alternatively, clinically significant anemia develops in immunosuppressed patients (eg, patients with HIV or organ transplant recipients) whose immune systems are unable to clear the infection or in patients with shortened RBC survival (eg, sickle cell anemia or hereditary spherocytosis). In the latter, the anemic presentation is termed an “aplastic crisis,” and often requires transfusion support.

Immunologic causes of acquired PRCA may be idiopathic or secondary to an underlying disease. PRCA develops in approximately 5% of patients with thymoma and, conversely, thymoma occurs in approximately 10% of patients presenting with PRCA. The response to thymectomy in these cases is variable; a minority of patients achieve complete remission after resection. PRCA may occur

Table 6-9 Classification of pure red cell aplasia

Congenital pure red cell aplasia
Diamond-Blackfan anemia
Acquired pure red cell aplasia
Primary pure red cell aplasia (likely immune-mediated mechanism)
Transient erythroblastopenia of childhood
Idiopathic
Secondary pure red cell aplasia (immune consequence of an underlying disorder)
Thymoma: post-ABO and autoimmune
Hematologic malignancies (eg, chronic lymphocytic leukemia, large granular lymphocyte leukemia, multiple myeloma)
Solid tumors (eg, stomach, breast, lung, renal cell carcinomas)
Infectious (eg, HIV, EBV, viral hepatitis)
Collagen vascular diseases
Drugs and chemicals (EPO antibodies may develop in those treated with ESAs)
Post-ABO incompatible bone marrow transplantation
Autoimmune chronic hepatitis or hypothyroidism
Parvovirus B19 (virus directly cytotoxic to red blood cell precursors)
Myelodysplastic syndrome (hematopoietic stem cell unable to differentiate along erythroid lineage)
EBV, Epstein-Barr virus.

in patients with underlying lymphoproliferative disorders (eg, large granular lymphocyte leukemia or chronic lymphocytic leukemia). Because large granular lymphocyte leukemia may be present even in the absence of significant lymphocytosis, it is recommended that patients with idiopathic PRCA undergo lymphocyte immunophenotyping to assess for this malignancy. In patients receiving ABO-mismatched bone marrow transplants, approximately 20% develop a prolonged RBC aplasia due to recipient isoagglutinins, especially anti-A, against donor RBCs. Generally, the condition improves over time or with the development of graft-vs-host disease. When the anemia is severe or life threatening, treatment with plasma exchange using donor-type plasma and high doses of recombinant human ESAs is effective in some patients.

Many different drugs have been reported to cause PRCA, and drug discontinuation may result in resolution. PRCA rarely has been described from development of anti-EPO antibodies during treatment with recombinant human ESAs, primarily after subcutaneous administration of Eprex (Janssen-Ortho, Toronto, Ontario, Canada), an EPO- α product marketed outside of the United States.

The number of ESA-associated PRCA cases peaked in 2001 and has since declined following changes in the manufacturing, distribution, storage, and administration of Eprex.

Diagnosis and treatment

Acquired PRCA presents with symptoms related to the severity of the anemia. Apart from pallor, physical examination in acquired primary PRCA often is normal. In acquired secondary PRCA, findings related to the underlying disease such as hepatomegaly, splenomegaly, or lymphadenopathy may be present.

Diagnosis of acquired PRCA is first suggested by finding a normochromic, normocytic, or macrocytic anemia with reticulocytopenia (absolute reticulocyte count of $<10,000/\mu\text{L}$). The white blood cell and platelet counts are generally normal. Bone marrow biopsy and aspirate establish the diagnosis. In parvovirus B19 infection, the marrow aspirate may show giant pronormoblasts. Routine karyotype and interphase fluorescence in situ hybridization panel for MDS should be included as part of the initial workup to evaluate for an underlying MDS. A careful history and physical exam should be used to guide further diagnostic testing. Additional studies to consider are a CT scan of the chest to evaluate for thymoma, EPO level, and parvovirus B19 DNA testing by polymerase chain reaction.

PRCA caused by parvovirus B19 in immunosuppressed individuals is treated with normal pooled serum IgG, which provides specific antibodies to clear the infection. PRCA associated with thymoma may respond to thymectomy. There does not appear to be any benefit to the removal of a normal thymus in patients with PRCA who do not have a thymoma or thymic hyperplasia identified.

Immunologically mediated PRCA is treated with sequential trials of immunosuppressive therapies (eg, prednisone, cyclosporine, oral cyclophosphamide, mycophenolate mofetil, horse antithymocyte globulin, alemtuzumab, rituximab), which ultimately lead to remission in 60% to 70% of patients. No prospective randomized clinical trial data exist to support the use of one immunosuppressive agent over another. Agent selection is based on the underlying disorder, if identified; and in idiopathic cases, prednisone or cyclosporine are typical first-line agents. A 3-month trial of each immunosuppressive agent is reasonable to assess for response to therapy. Responsive patients may be treated for 3 to 6 months before immunosuppression is slowly tapered. Many patients relapse after withdrawal of therapy and require a long-term approach to immunosuppression, particularly if an underlying disorder (lymphop-

roliferative disorder or collagen vascular disease) persists. Causes of death in nonresponding patients include infection, iron overload, or cardiovascular events.

Patients with severe symptomatic anemia are treated with transfusion therapy and face the associated risks of iron overload and alloantibody formation. Supplemental ESAs have been used in certain instances with variable success, such as post-ABO-incompatible bone marrow transplantation.

KEY POINTS

- PRCA is characterized by a severe normochromic, normocytic or macrocytic anemia with reticulocytopenia (absolute reticulocyte count of $<10,000/\mu\text{L}$).
- There are 3 pathophysiologic mechanisms of PRCA: immune-mediated, myelodysplasia, and parvovirus B19 infection in an immunocompromised host.
- Transient erythroblastopenia of childhood occurs in otherwise healthy infants and young children and typically resolves over several months. Treatment is supportive.
- Parvovirus B19 infection causes PRCA in all patients infected with the virus, but anemia only manifests in immunosuppressed patients or patients with shortened red cell survival (eg, sickle cell anemia, hereditary spherocytosis).
- PRCA secondary to parvovirus B19 infection is treated with intravenous immunoglobulin.
- In the absence of myelodysplasia or parvovirus B19 infection, PRCA is treated with immunosuppressive agents.

Anemia associated with liver disease

Patients with liver disease often have anemia and other hematologic abnormalities, with anemia reported in up to 75% of patients with chronic liver disease. The etiology of anemia is multifactorial, reflecting underproduction, blood loss, and increased RBC destruction. In alcoholic liver disease, concomitant folate deficiency may contribute and should be evaluated. Alcohol-induced pancreatitis may also lead to decreased vitamin B₁₂ absorption and subsequent deficiency. Ethanol and its metabolites have been shown to directly inhibit erythroid production and may lead to acute or chronic anemia, even in the absence of severe liver disease. EPO production and erythropoiesis are also suppressed by alcohol.

Viral hepatitis may be associated with PRCA. Combination therapy for chronic viral hepatitis may be complicated by clinically significant anemia secondary to ribavirin and/or interferon therapy. Ribavirin-induced hemolysis can be reversed by dose reduction or discontinuation. Interferon

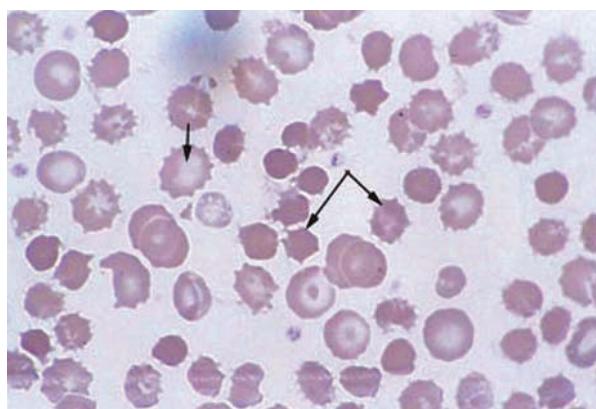
may contribute to anemia by inducing bone marrow suppression.

GI blood loss is common in patients with liver disease, especially those with esophageal varices. Shortened RBC survival is also noted in chronic liver disease and at least partially explained by congestive splenomegaly, abnormal erythrocyte metabolism, and alterations in RBC membrane lipids. Peripheral blood smear may demonstrate target cells or acanthocytes resulting from changes in cholesterol composition leading to alterations in RBC surface area. Spur cells (Figure 6-4), extreme forms of acanthocytes, may be present in persons with alcoholic liver disease, and are associated with a marked hemolytic anemia. In the presence of underlying cirrhosis, spur cell anemia is often irreversible without liver transplantation.

Anemia of liver disease is typically mild to moderate. It may become more severe as cirrhosis, portal hypertension, and splenomegaly develop. Anemia is often macrocytic, but MCV rarely exceeds 115 fL in the absence of megaloblastic changes within the bone marrow. The reticulocyte count is often minimally to moderately elevated, but may be suppressed by alcohol or concomitant iron deficiency. More marked reticulocytosis may be seen with hemorrhage or in patients with spur cell anemia. Peripheral blood smear shows acanthocytes and target cells as the disease severity increases. Bone marrow cellularity is often increased, and erythroid hyperplasia is observed. Megaloblastosis may be seen in up to 20% of subjects. Treatment of anemia in liver disease is primarily supportive. If present, iron, vitamin B₁₂, and folate deficiencies should be corrected. If persistent hemolysis is noted, folate supplementation should be continued. Alcohol and other toxins should be eliminated, when possible.

Figure 6-4 Spur cell anemia. Note the acanthocytes (also known as spur cells and indicated with arrows) and target cells.

Spur Cell Anemia



Sideroblastic anemias

Sideroblastic anemias are a heterogeneous group of congenital and acquired hematologic disorders characterized by the presence of ringed sideroblasts. Ringed sideroblasts are erythroid precursors with excess mitochondrial iron, in the form of ferritin, that surround (or ring) the nucleus. In both congenital and acquired sideroblastic anemia, formation of ringed sideroblasts is due to either insufficient production of protoporphyrin to utilize the iron delivered to erythroblasts or to defects in mitochondrial function affecting iron pathways and impairing its incorporation into protoporphyrin. In general, congenital sideroblastic anemias are microcytic, and acquired sideroblastic anemias are macrocytic.

Acquired sideroblastic anemias may be clonal (MDS; Chapter 17) or secondary to alcohol, drugs (eg, isoniazid, chloramphenicol, linezolid), or copper deficiency. Sideroblastic anemia associated with alcoholism is common and often found in severely malnourished persons with alcohol use and may be associated with folate deficiency. Pathogenesis is multifactorial and at least partially due to vitamin B₆ deficiency and/or ethanol-induced abnormalities in vitamin B₆ metabolism. Therefore, a trial of vitamin B₆ replacement is reasonable in affected persons. Vitamin B₆ therapy is effective treatment for X-linked congenital sideroblastic anemia as well.

Other underproduction anemias

The underproduction anemias discussed in this section are not typically differentiated by red cell size (MCV).

Copper deficiency anemia

Copper is an essential trace element that plays a critical role in numerous physiologic processes, including proliferation and differentiation. Copper deficiency is rare in humans. When present, it is typically due to either inadequate intake (eg, total parenteral nutrition without copper supplementation) or absorption (eg, postbariatric surgery, celiac disease, excessive zinc intake, congenital defect in copper transport, Menkes disease). Copper deficiency may cause anemia, neutropenia, and, less commonly, thrombocytopenia. A review of 40 patients with copper deficiency unrelated to Wilson disease found that 35% were postgastric resection, 25% postbariatric surgery, and an additional 30% had no identifiable cause. Anemia due to copper deficiency has no specific MCV and is reported variably as microcytic, normocytic, or macrocytic. It can mimic an acquired MDS, manifesting with a macrocytic anemia, neutropenia, and diverse marrow morphology including

ringed sideroblasts, dyserythropoiesis, dysmyelopoiesis, cytoplasmic vacuolization of erythroid and myeloid precursors, as well as hemosiderin-laden plasma cells. In addition to the hematologic manifestations, copper deficiency can cause neurologic symptoms resembling the subacute combined degeneration seen in patients with vitamin B₁₂ deficiency.

The mechanism by which copper deficiency results in hematologic changes is unknown. Copper is a cofactor for various redox enzymes, including hephaestin and ceruloplasmin, which are required to convert ferrous iron to its ferric form, a step necessary for the transport of iron by transferrin in the intestine and liver, respectively. Cytochrome c oxidase also requires copper as a cofactor. A decrease in this enzyme's activity may contribute to the development of ringed sideroblasts identified in some cases of copper deficiency. Measurement of serum copper level diagnoses copper deficiency; ceruloplasmin level can also be assessed but lacks specificity.

Anemia of cancer

Anemia in cancer patients is common, and its prevalence may exceed 90% in patients with advanced disease receiving chemotherapy. Its presence and severity is dependent on many variables, including cancer type and stage, as well as past and current therapy. Approximately two thirds of patients with cancer are anemic at diagnosis or become anemic (hemoglobin <12.0 g/dL) during the course of their treatment. The lowest hemoglobin levels are typically seen in patients with advanced disease and significantly compromised performance status. The mechanisms underlying anemia of malignancy are complex, with numerous factors contributing to its development. Cytokine-mediated changes cause both a relative decrease in EPO production and a decrease in EPO responsiveness of erythroid precursors. As in AOCD, cytokines promote hepcidin production resulting in iron-restricted erythropoiesis. Hemoglobin concentrations in cancer patients inversely correlate with inflammatory markers, serum hepcidin, serum ferritin, EPO, and reactive oxygen species. Additional factors contributing to anemia of cancer include bone marrow infiltration, treatment effects of chemotherapy and radiotherapy, blood loss, autoimmune and microangiopathic hemolysis, and nutritional deficiencies.

Supportive care of cancer patients has changed with the availability of recombinant ESAs, which both decrease transfusion requirements and may also improve overall health-related quality of life. ESAs may, however, cause tumor growth in some patients, and clinical studies have shown shortened survival in those with advanced breast,

head and neck, lymphoid, and non–small cell lung cancer, especially when a hemoglobin concentration of 12 g/dL was targeted. A meta-analysis that analyzed 13,933 cancer patients treated on 53 randomized controlled trials using ESAs vs standard of care demonstrated a 17% increase in mortality in ESA-treated patients during the active study period. A 10% increase in mortality persisted when analysis was restricted to studies of patients treated with chemotherapy. Reanalysis of the same data showed that ESAs do not increase risk of tumor progression if administered according to published guidelines, though a small increased risk of venous thromboembolic disease persists. The American Society of Hematology/American Society of Clinical Oncology guidelines on ESA use in cancer patients recommend using the lowest possible ESA dose with the goal of gradually increasing hemoglobin concentration to a level that decreases the need for transfusion support while still remaining <12 g/dL. Notably, ESAs are not recommended for patients receiving chemotherapy with curative intent and should not be given in cancer patients not receiving concurrent myelosuppressive chemotherapy. (Patients with low-risk MDS are an exception to this recommendation.) Combining an ESA with intravenous rather than oral iron increases the response rate with no increase in complications.

KEY POINTS

- Anemia is frequent in cancer patients and leads to decreased quality of life.
- ESAs reduce transfusion requirements and may improve quality of life in cancer patients.
- The use of ESAs in cancer patients requires careful patient counseling regarding potential benefits and risks, and their use should follow published guidelines.

Myelophthusic anemia

Myelophthusic anemia is a normochromic, normocytic anemia that occurs when normal marrow space is infiltrated and replaced by abnormal or nonhematopoietic cells. The term *myelophthusic* is not commonly used in clinical practice and more often this anemia is referred to descriptively as a marrow infiltrative process. Causes include tumors, granulomatous disorders, bone marrow fibrosis (due to a primary hematologic or nonhematopoietic disorder), and lipid storage diseases; all causes may induce secondary marrow fibrosis. The peripheral blood smear in myelophthusic anemia shows a leukoerythroblastic process with

teardrop-shaped and nucleated RBCs, immature leukocytes, and occasional myeloblasts. Rarely, carcinocythemia, defined as cancer cells within the circulating blood, is seen. Bone marrow biopsy may show frank tumor cells, Gaucher disease, or other infiltrating disorders, as well as marked marrow fibrosis. These conditions may be accompanied by extramedullary hematopoiesis resulting in organomegaly due to marrow elements in the spleen, liver, or other affected tissues. T1-weighted MRI may demonstrate areas of abnormal signal, consistent with marrow infiltration. Treatment is directed at the underlying disease.

During infancy, anemia secondary to marrow fibrosis may be seen in the setting of osteopetrosis or marble bone disease, which is caused by failure of osteoclast development or function. These conditions vary in their severity, but infants affected with the autosomal recessive form present within the first few months of life with pancytopenia, hepatosplenomegaly, cranial nerve palsies, and changes in calcium levels. Mutations in at least ten genes have been identified in patients with osteopetrosis, accounting for 70% of cases. Severe cases are treated by bone marrow transplantation.

Anemia from malnutrition or anorexia nervosa

Prolonged starvation can lead to a normochromic, normocytic anemia, and bone marrow aspirates from such patients are often hypocellular. Rarely, patients with severe starvation or anorexia nervosa can have gelatinous transformation of the marrow with few marrow-derived cells seen histologically (Figure 6-5).

Anemia associated with endocrine disorders

In general, the anemia associated with endocrine disorders is mild and the symptomatology overshadowed by the clinical effects of the specific hormone deficiency. In some cases, the anemia may be physiologic due to the decreased oxygen requirements accompanying the hormone deficiency.

Deficiencies in hormones produced by the anterior lobe of the pituitary gland (thyroid hormone, androgens, or cortisol), which modulate EPO production, are associated with a mild normochromic, normocytic anemia. The bone marrow is usually hypoplastic and resembles that seen in other marrow failure states. The anemia improves after initiation of appropriate hormone replacement to address the underlying deficiency.

Patients with primary hypothyroidism may be anemic due to an absence of EPO-stimulated erythroid colony formation from lack of triiodothyronine, thyroxine, and re-

Anorexia Nervosa - Marrow Morphology

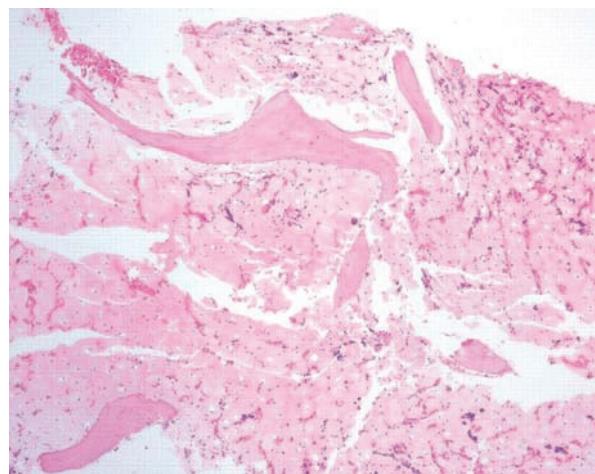


Figure 6-5 Anorexia nervosa. A marrow biopsy is shown, illustrating almost complete replacement of the marrow by hyaluronic acid extracellular matrix material. Hematopoietic elements and fat cells are markedly decreased. Hematoxylin and eosin stain; magnification $\times 4$.

verse triiodothyronine. The anemia is usually normochromic and normocytic, and the hemoglobin concentration typically does not fall below 8 g/dL. Macrocytosis may be present in patients with autoimmune hypothyroidism, particularly if there is coexistent vitamin B₁₂ or folate deficiency, or hemolysis. Conversely, microcytosis can occur in women with concomitant iron deficiency from abnormal uterine bleeding, which can occur in myxedema. There is a well-recognized association between autoimmune thyroid disease and PA, so patients with either disorder should be screened for the other. Response to thyroid replacement is typically slow, and it may take months before the anemia resolves. Concurrent administration of thyroid replacement and oral iron therapy can affect absorption. Therefore, stable and consistent dosing should be maintained or intravenous iron therapy should be considered. Microcytic anemia in patients with hyperthyroidism is also described and often corrects when patients become euthyroid.

Hypogonadism usually results in a decrease of 1 to 2 g/dL in hemoglobin concentration due to androgens' role in stimulating EPO production and increasing its effects on the developing erythron. This mechanism explains why men have higher hemoglobin concentrations compared to age-matched women. Men treated with antiandrogen therapy for prostate cancer therefore typically have a decrease in hemoglobin concentration by 1 to 2 g/dL.

A normochromic, normocytic anemia responsive to ESAs or glucocorticoids may be seen in patients with Addison disease. These patients develop a mild decrease in RBC mass that may be unrecognized due to a concomitant decrease in plasma volume, resulting in a normal hemoglobin concentration. When glucocorticoid therapy replacement is initiated, plasma volume is restored and the anemia is unmasked. Androgens may be useful to correct the anemia of myelofibrosis and myeloid metaplasia.

Anemia presents earlier and is more severe in patients with diabetic nephropathy compared to patients with other causes of renal failure. The exact mechanism for this finding remains unclear.

Anemia associated with pregnancy

Anemia is a common complication of pregnancy. RBC mass increases 20% to 30% during gestation, while plasma volume increases 40% to 50%, resulting in a normochromic and normocytic anemia. This physiologic anemia of pregnancy reaches a nadir at approximately 30 weeks gestational age. Plasma volume expansion plateaus at 30 weeks while RBC mass continues to rise, so the hematocrit may increase somewhat during the final 10 to 12 weeks of pregnancy. A large study of pregnancy in Australia with over 124,000 individuals found that 7% of women were anemic. Anemia of pregnancy was associated with a higher risk of fetal distress and perinatal complications but not with the infant's subsequent development.

Definitions of pathologic anemia during pregnancy vary. United Kingdom guidelines define it as a hemoglobin concentration <11 g/dL in the first trimester, <10.5 g/dL in the second and third trimesters, and <10 g/dL in the postpartum period. Evaluation and workup in pregnant patients should be similar to nonpregnant patients. Special consideration should be paid to any proposed therapies, given potential effects on both the mother and fetus. Anemia of pregnancy can be exacerbated in individuals with sickle cell disease and thalassemia and needs to be carefully monitored and managed.

Iron deficiency during pregnancy is common, especially in non-Western cultures. A full-term pregnancy requires 1 g of iron; 300 mg for the fetus, 200 mg to replace maternal iron losses, and 500 mg for the expanding maternal RBC mass. Postpartum iron is secreted in breast milk to nourish the developing infant. Folate requirements also increase during pregnancy. Megaloblastic anemia has been reported, predominantly during the third trimester when maternal folate stores become wasted. Prenatal vitamins containing both iron and folate can help reduce, but not eliminate, these risks.

Vitamin B₁₂ deficiency rarely occurs during pregnancy. Serum vitamin B₁₂ levels may be less reliable during pregnancy because of changes in protein binding, and MMA levels should be checked to confirm true deficiency. There are reports of idiopathic acquired aplastic anemia patients experiencing a worsening in their cytopenias or even relapse during pregnancy.

KEY POINTS

- Anemia in pregnancy is due in part to an imbalance between expansion of plasma volume and the RBC mass.
- Iron deficiency and folate deficiency are important causes of anemia in pregnancy, with iron deficiency being the most common.
- The evaluation of anemia in pregnancy should be similar to the evaluation of anemia in nonpregnant individuals.

Anemia in the elderly

Approximately 11% of men and 10% of women over age 65 years are anemic (defined as hemoglobin concentration <13 g/dL for men; <12 g/dL for women). Prevalence rates are higher in elderly African Americans and increase with age (30% in patients over 80 years; 37% in those over 90 years). In this population, anemia is an independent risk factor for cognitive decline and is associated with decreased bone density, muscle strength, and physical performance. The presence of anemia, with or without other comorbid diseases, is associated with increased hospitalization, morbidity, and mortality.

Analysis of NHANES III data found that approximately two thirds of anemia cases were attributable to iron deficiency, other nutritional deficiencies, chronic inflammatory illnesses, and/or renal insufficiency. Roughly 34% of cases were unexplained but are likely due to a variety of underappreciated etiologies such as underlying renal disease, low EPO, low androgen, and/or alterations in bone marrow stem cells and cellularity.

Due to comorbid conditions typically present in the elderly population, it is difficult to reach consensus on goal hemoglobin concentration levels to target for supportive blood transfusion therapy. However, many attempt to maintain a hemoglobin of 9 to 10 g/dL. As anemia in an elderly patient is often multifactorial, a thorough clinical and laboratory evaluation is justified to identify those causes of anemia that are amenable to therapy. A reasonable approach to evaluation is given in Table 6-10.

Table 6-10 Practical approach for the evaluation of anemia in the elderly

Initial assessment
1. Anemia-oriented clinical history and physical examination, with emphasis on comorbid conditions and medications
2. CBC/differential/platelet, absolute reticulocyte count, smear review
3. Iron panel (Fe, TIBC, ferritin)
4. Serum cobalamin and folate levels, RBC folate (methylmalonic acid, serum homocysteine)
5. Chemistry panel (including calculated creatinine clearance)
6. Thyroid function (TSH)
Additional assessment, if indicated
1. Serum testosterone
2. Serum EPO
3. Laboratory assessment of inflammation (ESR, C-reactive protein)
4. Bone marrow aspiration and biopsy, cytogenetics

Modified from Guralnik JM et al, *Hematology Am Soc Hematol Educ Program*. 2005;528–532.

TSH, thyroid-stimulating hormone.

KEY POINTS

- Anemia is common in elderly patients and often multifactorial.
- In two thirds of elderly patients, the anemia is caused by nutritional deficiency or AOCD. It is unexplained in one third of patients.
- Functional impairment and increased morbidity and mortality have been demonstrated in anemic elderly patients.
- Transfusion practice to maintain hemoglobin concentration thresholds of 9 to 10 g/dL for the elderly population may be prudent.

Anemia associated with HIV infection

Anemia is the most common hematologic abnormality associated with HIV infection, and its prevalence increases with HIV disease progression. Anemia is associated independently with decreased survival and decreased quality of life in HIV-infected patients. Anemia in this population is multifactorial, and the most likely etiologies depend on both the stage of the infection and the medications the patient is receiving.

The underlying inflammatory pathways of HIV contribute to the pathophysiology of anemia. In addition, antiretroviral therapies as well as drugs used for prophylaxis

or treatment of opportunistic infections, may result in bone marrow suppression. Zidovudine (AZT) is the leading cause of therapy-associated anemia due to bone marrow suppression among patients with HIV and was reported in up to 25% of patients in phase 1 trials. Macrocytosis is also common in patients receiving AZT. Rarely, tenofovir is associated with anemia or other hematologic side effects. Macrocytosis has been reported in stavudine and lamivudine. Trimethoprim-sulfamethoxazole, ganciclovir, valganciclovir, and Amphotericin B can also result in bone marrow suppression. Infections commonly seen in HIV patients and associated with anemia include *Mycobacterium avium* complex, tuberculosis, histoplasmosis, cytomegalovirus, Epstein-Barr virus, and human parvovirus (see the section “Acquired pure red cell aplasia”). Malignant disorders, mainly non-Hodgkin lymphoma, can infiltrate the bone marrow and cause anemia. Nutritional deficiencies, including vitamin B₁₂, folate, and iron, are common in HIV patients and are related to blood loss, malabsorption, and overall poor nutrition. These patients are also at risk for hemolysis, including microangiopathic hemolysis, antibody-mediated mechanisms, and drug-induced mechanisms, especially in patients with glucose-6-phosphate dehydrogenase deficiency. Hypogonadism is a frequent finding in patients with advanced HIV and is associated with a mild anemia as described previously. The HIV virus itself also directly infects bone marrow cells and may interfere with hematopoiesis.

The use of highly active antiretroviral therapy (HAART) has been shown to reduce the prevalence of anemia in several population studies, even when zidovudine remains within the regimen. In addition to HAART, the management of anemia in HIV patients should include correction of nutritional deficiencies and appropriate prevention and treatment of infections. ESAs reduce transfusion requirements in HIV patients with baseline EPO levels of <500 mU/mL, in whom nutritional deficiencies and other causes have been corrected.

KEY POINTS

- HIV-related anemia is common and independently associated with decreased survival.
- Anemia in HIV is multifactorial and may reflect viral infection, malignancy, malnutrition, and medication effect.
- In patients treated with zidovudine, the finding of macrocytosis is more common than anemia.
- HAART reduces the incidence and degree of anemia in HIV-infected patients.

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Thalassemia, sickle cell disease, and other hemoglobinopathies

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Introduction

Hemolysis is the accelerated destruction of red blood cells (RBCs), leading to decreased RBC survival. The bone marrow's response to hemolysis is increased erythropoiesis, reflected by reticulocytosis. As is typical in hemoglobinopathies, the bone marrow is unable to completely compensate for hemolysis, leading to anemia.

Abnormalities of hemoglobin

Hemoglobin is the oxygen-carrying protein within RBCs. It is composed of 4 globular protein subunits, called globins, each with an oxygen-binding heme group. The 2 main types of globins are the α -globins and the β -globins, which are made in essentially equivalent amounts in precursors of RBCs. Normal adult hemoglobin (HbA) has 2 α -globins and 2 β -globins ($\alpha_2\beta_2$). Genes on chromosomes 16 and 11 encode the α -globins and β -globins, respectively. There are also distinct embryonic, fetal, and minor adult analogues of the α -globins and β -globins encoded by separate genes.

Hemoglobin production

The α -globin gene cluster is on chromosome 16 and includes the embryonic ζ -globin gene and the duplicated α -globin genes (α_1 and α_2), which are expressed in both fetal and adult life. The β -globin gene cluster is on chromosome 11 and includes an embryonic ϵ -globin gene, the 2 fetal γ -globin genes ($\text{A}\gamma$ and $\text{G}\gamma$), and the 2 adult δ - and β -globin genes. Both clusters also contain non-functional genes (pseudo-genes) designated by the prefix ψ . The θ -globin gene downstream of α_1 has unknown functional significance.

The expression of each globin gene cluster is under the regulatory influence of a distant upstream locus control region (LCR). The LCR for the β -cluster resides several kilobases upstream. A similar regulatory region, called HS-40, exists upstream of the α cluster. The LCRs contain DNA sequence elements that interact with erythroid-specific and nonspecific DNA-binding proteins. LCRs serve as a "master switch," by inducing expression of downstream gene clusters. LCRs also facilitate the binding and interaction of transcriptional regulatory proteins in proximity to the specific genes within the downstream cluster. These regulatory proteins influence the promoter function of the α -globin and

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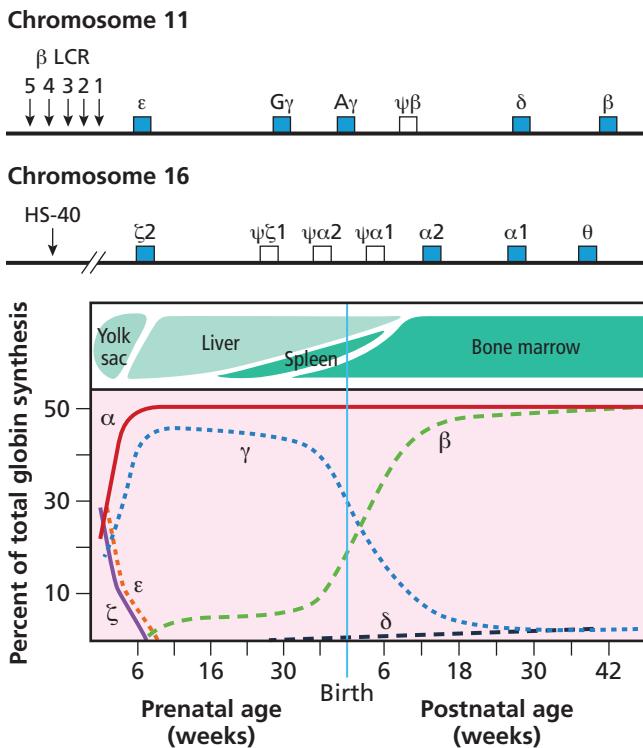


Figure 7-1 Hemoglobin gene clusters and developmental hematopoiesis. The organization of the α - and β -globin gene clusters are shown at the top of the figure. The bottom portion of the figure illustrates the developmental changes in Hb production, both by the site of production of blood and changes in the proportions of the different globins. Modified with permission from Stamatoyannopoulos G et al, eds. The Molecular Basis of Blood Diseases. 3rd ed. Philadelphia, PA: W. B. Saunders; 2001.

β -globin genes to achieve a high level of erythroid- and development-specific gene expression.

Figure 7-1 details the organization of the α - and β -clusters with the associated upstream regulatory elements and the normal hemoglobin species produced during the developmental stages from intrauterine to adult life. Note that the genes are expressed developmentally in the same sequence in which they are organized physically in these clusters (left to right; 5' to 3'). The process of developmental changes in the type and site of globin gene expression is known as hemoglobin switching. Switching within the cluster is influenced by differential enhancing and gene-silencing effects imparted by the combination of the LCR and local regulatory proteins, but the entire process of regulatory determination remains incompletely defined. The ability to modulate the switch from the synthesis of γ - to β -globin chains has long been of interest because “reversing the switch” to enhance expression of fetal hemoglobin (HbF) could successfully treat sickle cell disease. A number

of different transcription factors have been identified, including MYB, KLFs, BCL11A, and SOX6, which repress fetal globin gene expression in erythroid cells. Turning off these repressors could increase fetal hemoglobin expression and treat hemoglobinopathies.

Hemoglobin structure

Hemoglobin is a tetramer consisting of 2 pairs of globin chains. Heme, a complex of ferrous iron and protoporphyrin, is linked covalently to each globin monomer and can reversibly bind 1 oxygen molecule. The molecular mass of hemoglobin is approximately 64 kDa. The α -chains contain 141 amino acids, and the β -chains contain 146 amino acids, as do the β -like globins, δ and γ , which differ from β by 10 and 39 amino acids, respectively. The compositions of normal Hb species throughout development are depicted in Figure 7-1. The postembryonic hemoglobins are HbA ($\alpha_2 \beta_2$), HbA₂ ($\alpha_2 \delta_2$), and HbF ($\alpha_2 \gamma_2$).

When hemoglobin is deoxygenated, there are substantial changes in the structures of the individual globins and the hemoglobin tetramer. The iron molecule rises from the plane of its heme ring, and there is a significant rotation of each globin chain relative to the others. In the deoxy conformation, the distance between the heme moieties of the β -chains increases by 0.7 nm. This conformation is stabilized in a taut (T) conformation by salt bonds within and between globin chains and by the binding of allosteric modifiers such as 2,3-bisphosphoglycerate (2,3-BPG) and of protons. The binding of oxygen to hemoglobin leads to disruption of the salt bonds and transition to a relaxed (R) conformation.

Hemoglobin function

Hemoglobin enables RBCs to deliver oxygen to tissues by its reversible binding of oxygen. With the sequential binding of 1 oxygen molecule to each of the 4 heme groups, the salt bonds are progressively broken, which increases the oxygen affinity of the remaining heme moieties. Cooperation between the heme rings results in the characteristic sigmoid-shaped oxygen affinity curve. This phenomenon accounts for the release of relatively large amounts of oxygen with small decreases in oxygen tension.

Deoxygenation of hemoglobin is modulated by certain biochemical influences. For example, deoxyhemoglobin binds protons with greater avidity than oxyhemoglobin, which results in a direct dependence of oxygen affinity on pH over the physiologic pH range. This Bohr effect has 2 major physiologic benefits: (i) the lower pH of metabolically active tissue decreases oxygen affinity, which accommodates oxygen delivery; and (ii) the higher pH level resulting from carbon dioxide elimination in the lungs in-

creases oxygen affinity and oxygen loading of RBCs. An additional important influence on oxyhemoglobin dissociation is temperature. Hyperthermia decreases affinity, providing the opportunity to deliver more oxygen at the tissue level. 2,3-BPG, a metabolic intermediate of anaerobic glycolysis, is another physiologically important modulator of oxygen affinity. When 2,3-BPG binds in the pocket formed by the amino termini of the β -chains, it stabilizes the deoxy conformation of hemoglobin, thereby reducing its oxygen affinity. The intraerythrocytic molar concentrations of 2,3-BPG and hemoglobin are normally about equal (5 mM). When 2,3-BPG levels increase during periods of hypoxia, anemia, or tissue hypoperfusion, oxygen unloading to tissues is enhanced.

Carbon dioxide reacts with certain amino acid residues in the β -chain of hemoglobin; however, this does not play a significant role in carbon dioxide transport. It recently has been reported that hemoglobin binds nitric oxide in a reversible manner. The participation of hemoglobin in modifying regional vascular resistance through this mechanism has been proposed.

Disorders of hemoglobin

Disorders of hemoglobin can be classified as quantitative or qualitative. Quantitative hemoglobin disorders result from the decreased and imbalanced production of generally structurally normal globins. For example, if β -globin production is diminished by a mutation, there is a relative excess of α -globin chains. Such imbalanced production of α - and β -globin chains damages RBCs and their precursors in the bone marrow. These quantitative hemoglobin disorders are called thalassemias. Qualitative abnormalities of hemoglobin arise from mutations that change the amino acid sequence of the globin, thereby producing structural and functional changes in hemoglobin. There are 4 ways in which hemoglobin can be qualitatively abnormal: (i) decreased solubility (eg, HbS), (ii) instability (eg, Hb Koln), (iii) altered oxygen affinity (eg, Hb M-Saskatoon), and (iv) altered maintenance of the oxidation state of the heme-coordinated iron (eg, Hb M-Iwate). Hemolytic anemia results from decreased solubility and instability of hemoglobin. Qualitative hemoglobin disorders often are referred to as hemoglobinopathies, even though the term technically can apply to both qualitative and quantitative disorders. Both qualitative and quantitative disorders of hemoglobin can be subdivided by the particular globin that is affected; for example, there are α -thalassemias and β -hemoglobinopathies, among others. We begin this chapter with a review of the thalassemias and end the section with a discussion of several of the common qualitative hemoglobin disorders.

Thalassemia

CLINICAL CASE



A healthy 48-year-old female of African descent is referred to you for evaluation of refractory microcytic anemia. She has been treated with oral iron formulations many times throughout her life. Hemoglobin values have always ranged from 10 to 11 g/dL with a mean corpuscular volume (MCV) ranging from 69 to 74 fL. She has no other prior medical history. Her examination is entirely unremarkable. Peripheral blood smear is significant for microcytosis, mild anisopoikilocytosis, and a small number of target cells. The hemoglobin concentration is 10 g/dL with an MCV of 71 fL and mean corpuscular hemoglobin (MCH) of 23 pg. Additional laboratory studies include a transferrin saturation of 32% and a normal ferritin of 285 ng/mL. Hemoglobin electrophoresis reveals hemoglobin A 98% and hemoglobin A2 1.8%.

Thalassemia occurs when there is quantitatively decreased synthesis of often structurally normal globin proteins. Mutations that decrease the synthesis of α -globins cause α -thalassemia; mutations that decrease the synthesis of β -globins cause β -thalassemia. Heterozygous thalassemia (thalassemia trait) appears to confer protection against severe *Plasmodium falciparum* malarial infection. As a result of this selective advantage, a wide variety of independent mutations leading to thalassemia have arisen over time and have been selected for in populations residing in areas where malaria is (or once was) endemic. In general, α -thalassemias are caused by deletions of DNA, whereas β -thalassemias are caused by point mutations.

The major result of a deletion or mutation in all forms of thalassemia is decreased or absent production of one or more globin chains. This results in unbalanced synthesis of individual alpha and beta subunits. Unpaired α - or β -globin chains are insoluble or form tetramers that do not release oxygen readily and precipitate in the red cells (eg, α_4, β_4). For example, if β -globin synthesis is reduced by a mutation, there is a relative excess of α -globin chains. Such imbalanced production of α - and β -globin chains results in damage to RBC precursors in the bone marrow. This damage occurs largely because the excess unpaired globin is unstable, and precipitates within early RBC precursors in the bone marrow and oxidatively damages the cellular membrane. If the α - and β -globin imbalance is severe, most of the RBC precursors in the bone marrow are destroyed before they can be released into the circulation. A severe microcytic anemia results. The body attempts to compensate for the anemia by increasing erythropoietic

activity throughout the marrow and sometimes in extramedullary spaces, although this effort is inadequate and compensation is incomplete. This pathophysiologic process is called ineffective erythropoiesis. The complications of thalassemia vary and depend on the severity of the chain imbalance and identity of the globin chain.

The thalassemias can be described simply by 2 independent nomenclatures: genetic and clinical. The genetic nomenclature denotes the type of causative mutation, such as α -thalassemia or β -thalassemia. The clinical nomenclature divides the thalassemias into the asymptomatic trait state (thalassemia minor), severe transfusion-dependent anemia (thalassemia major), and everything in between (thalassemia intermedia). The 2 systems can be used together, giving α -thalassemia minor or β -thalassemia intermedia, for example. More recently, a new clinical classification based on transfusion dependence has been introduced and divides patients into either having transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT).

β -Thalassemias

β -Thalassemia is prevalent in the populations where malaria was once endemic, such as the Mediterranean region, the Middle East, India, Pakistan, and Southeast Asia, and is somewhat less common in Africa. It is rarely encountered in Northern European Caucasians. However, due to migration, thalassemia (both α - and β -thalassemia) is now found in most regions of the world, including North America.

Molecular basis

β -Thalassemia results from >200 different mutations in the β -globin gene complex (Figure 7-2). Abnormalities have been identified in the promoter region, mRNA cap site, 5' untranslated region, splice sites, exons, introns, and polyadenylation signal region of the β -gene. Gene deletions are infrequent except in $\delta\beta$ - and $\epsilon\gamma\delta\beta$ -thalassemias. A variety of single-base pair mutations or insertions or deletions of nucleotides represent the majority of described mutations. Thus, defects in transcription, RNA processing, and translation or stability of the β -globin gene product have been observed. Mutations within the coding region of the globin gene allele may result in nonsense or truncation mutations of the corresponding globin chain, leading to complete loss of globin synthesis from that allele (β^0 -thalassemia allele). Alternatively, abnormalities of transcriptional regulation or mutations that alter splicing may cause mild to markedly decreased, but not absent, globin gene synthesis (β^+ -thalassemia allele). β -thalassemia major (Cooley's anemia) and β -thalassemia intermedia can be due

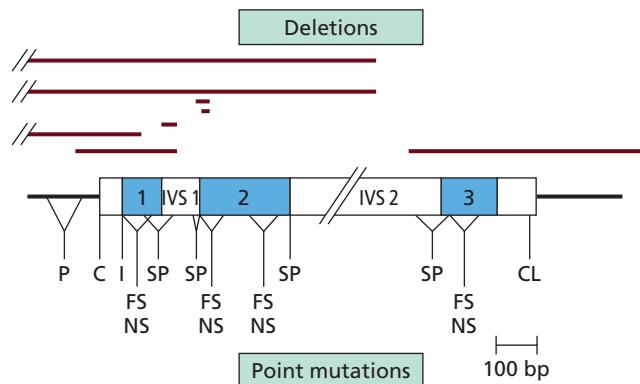


Figure 7-2 Common β -thalassemia mutations. The major classes and locations of mutations that cause β -thalassemia are shown. Redrawn from Stamatoyannopoulos G et al., eds. The Molecular Basis of Blood Diseases. 3rd ed. Philadelphia, PA: WB Saunders; 2001. C, cap site; CL, RNA cleavage [poly(A)] site; FS, frameshift; I, initiation codon; NS, nonsense; P, promoter boxes; SP, splice junction, consensus sequence, or cryptic splice site.

to various genotypes, including homozygosity or compound heterozygosity for 2 β^0 alleles (β^0/β^0) or compound heterozygosity with a β^0 and β^+ allele (β^0/β^+). Patients with β -thalassemia trait are generally heterozygous, carrying a single β -thalassemia allele (β/β^0 , β/β^+), but some patients who are homozygous or compound heterozygous for 2 very mild β^+ alleles may also have β -thalassemia minor phenotype. The clinical phenotype of patients with β -thalassemia is heterogeneous and is determined primarily by the globin chain imbalance due to the number and severity of the abnormal alleles inherited. Coinheritance of other abnormalities affecting α - or γ -globin synthesis or structural abnormalities of hemoglobin (eg, HbC, HbE) also affects the chain imbalance and hence the clinical phenotype. For example, patients with β/β^0 or β/β^+ mutations may present with a phenotype of β -thalassemia intermedia if alpha triplication or quadriplication is present, leading to further imbalance in the alpha-to-beta globin ratio. Secondary genetic modifiers, such as uridine diphosphateglucuronosyltransferase gene polymorphisms, also contribute to the overall phenotype.

Pathophysiology

The defect in β -thalassemia is a reduced or absent production of β -globin chains with a relative excess of α -chains. The decreased β -chain synthesis leads to impaired production of the $\alpha_2\beta_2$ tetramer of HbA, decreased hemoglobin production, and an imbalance in globin chain synthesis. The reduction in HbA in each of the circulating RBCs results in hypochromic, microcytic RBCs with target cells, a characteristic finding in all forms of β -thalassemia. Ag-

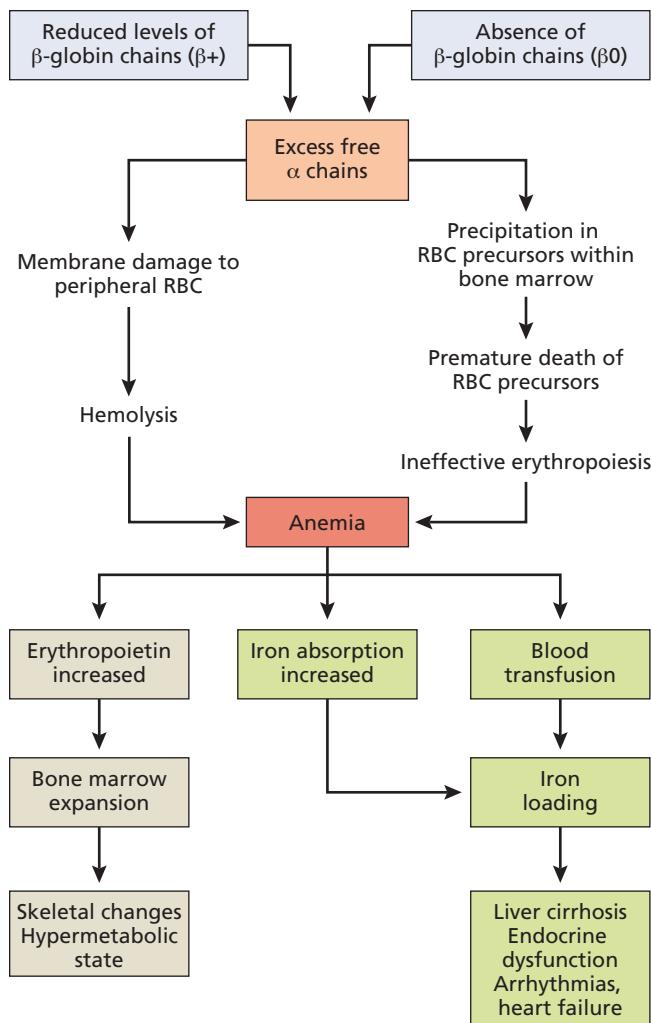


Figure 7-3 Pathophysiology of β-thalassemia. Effects of excess production of free α-globin chains in β-thalassemia. Adapted with permission from Viprakasit V and Origa R. In: Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT). 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation; 2014.

gregates of excess α-chains precipitate and form inclusion bodies, leading to premature destruction of erythroid precursors in the bone marrow (ineffective erythropoiesis) (Figure 7-3). In more severe forms, circulating RBCs may also contain inclusions, leading to early clearance by the spleen. The precipitated α-globin chains and products of degradation may also induce changes in RBC metabolism and membrane structure, leading to shortened RBC survival. The response to anemia and ineffective erythropoiesis is increased production of erythropoietin leading to erythroid hyperplasia, which can produce skeletal abnormalities, splenomegaly, extramedullary masses and osteoporosis. Ineffective erythropoiesis is associated with increased gastrointestinal iron absorption due to decreased

hepcidin. RBC membrane damage with increased surface expression of anionic phospholipids, platelet activation, and changes in hemostatic regulatory proteins contribute to a hypercoagulable state in thalassemia, which is worsened after splenectomy.

α-Thalassemias

There is a high prevalence of α-thalassemia in areas of the Old World where malaria was once endemic, including Africa, the Mediterranean region, Southeast Asia, and, to a lesser extent, the Middle East.

Molecular basis

Two copies of the α genes are normally present on each chromosome 16, making the defects in α-thalassemia more heterogeneous than in β-thalassemia (Figure 7-4). The α⁺-thalassemias result from deletion of one of the linked genes, $-α/αα$, or impairment due to a point mutation, designated $α^Tα/αα$. The deletion type of α⁺-thalassemia is due to unequal crossover of the linked genes, whereas the nondeletion type includes mutations resulting in abnormal transcription or translation or the production of unstable α-globin. The $-α/αα$ genotype (the “silent carrier” state) occurs in approximately 1 in 3 African Americans. Hemoglobin Constant Spring is one example of many nondeletional α-thalassemias. It is a nondeletional α⁺-thalassemia, common in Southeast Asia, resulting from a mutation that affects termination of translation and results in abnormally elongated α-chains. The $--/αα$ genotype ($α^0$ -thalassemia) of α-thalassemia trait due to loss of linked α-genes on the same chromosome (*cis* configuration), is more common in individuals of Asian descent, whereas the $-α/-α$ genotype (deletions in the *trans* position) is more common in individuals of African or Mediterranean descent.

Pathophysiology

As in the β-thalassemias, the imbalance of globin chain synthesis results in decreased hemoglobin synthesis and microcytic anemia. Excess γ- and β-chains form tetramers termed Hb Bart and HbH, respectively. These tetramers are more soluble than unpaired α-globins (as in β-thalassemia) and form RBC inclusions slowly. Consequently, although α-thalassemia is associated with a hemolytic anemia, it does not lead to significant ineffective erythropoiesis. The homozygous inheritance of α⁰-thalassemia ($--/--$) results in the total absence of α-chains, death in utero, or hydrops fetalis. Unpaired γ-chains form Hb Bart ($γ_4$), and there may be persistence of embryonic hemoglobins. Hb Bart is soluble and does not precipitate; however, it has a very high oxygen affinity and is unable to deliver oxygen to the tissues. This leads to severe fetal tissue

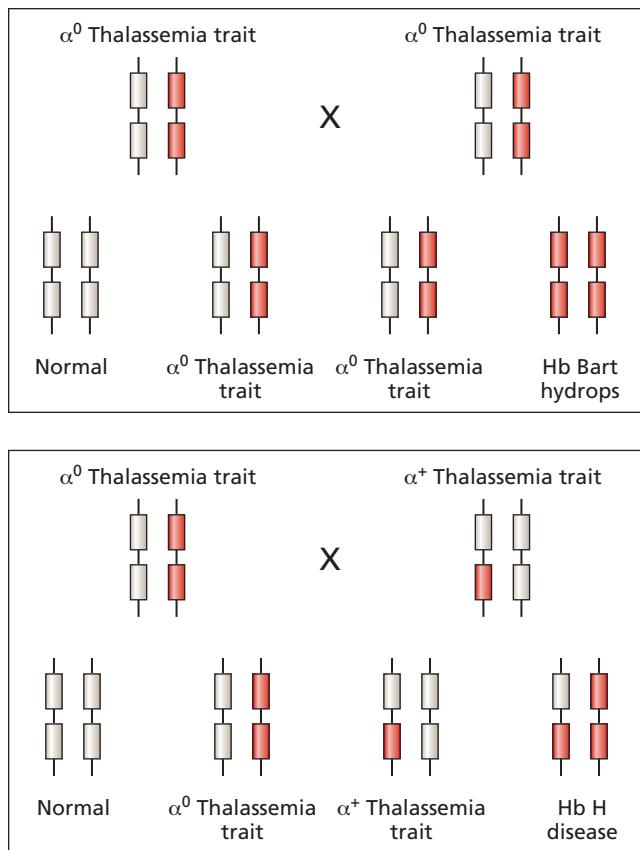


Figure 7-4 Genetics of α -thalassemia. The α -globin genes are represented as boxes. The red α -globin genes represent deletions or otherwise inactivated α -genes. The open boxes represent normal α -genes. The terms α^0 - and α^+ -thalassemia are defined in the text. The potential offspring of 2 parents with α^0 -thalassemia trait is shown in the upper panel. The potential offspring of 1 parent with α^0 -thalassemia trait and the other with α^+ -thalassemia trait is shown in the lower panel (note the lack of Hb Bart's hydrops fetalis in these offspring). Redrawn from Stamatoyannopoulos G et al., eds. The Molecular Basis of Blood Diseases. 3rd ed. Philadelphia, PA: W. B. Saunders; 2001.

hypoxia, resulting in edema, congestive heart failure, and death. HbH disease results from the coinheritance of α^0 -thalassemia and α^+ -thalassemia ($--/\alpha$) or α^0 -thalassemia and a nondeletional form of α -thalassemia ($--/\alpha^T\alpha$) such as Hb Constant Spring ($--/\alpha^{CS}\alpha$). The excess β -chains form HbH (β_4) that is unstable, causing precipitation within circulating cells and hemolysis. Patients have moderately severe hemolytic anemia.

HbH also can be produced as an acquired phenomenon in the setting of myelodysplastic syndromes and some myeloid leukemias, in which somatic mutations of the *ATRX* gene downregulate α -globin production and cause globin chain imbalance. This condition is called the α -thalassemia–myelodysplastic syndrome. The X-linked *ATRX*

gene encodes a chromatin-remodeling factor (X-linked helicase 2) that regulates α -globin production. Constitutional deletions of this gene produce the α -thalassemia–mental retardation syndrome.

Clinical classification of thalassemia

The clinical severity of thalassemia is highly variable. Prior to 2012, clinically significant β -thalassemia was primarily classified phenotypically into β -thalassemia major (β TM) or β -thalassemia intermedia (β TI). β TM represents patients with severe anemia and transfusion dependence early in life, while β TI represents a more heterogeneous group of mild, moderate to severe anemia with varying transfusions needs. In the past, management guidelines focused primarily on β TM, and β TI was considered mild due to higher hemoglobin values and fewer transfusion requirements. However, more recently, it has been shown that patients with β TI can indeed develop major complications later in life and thus need closer monitoring and aggressive intervention earlier in the course of the disease.

The new clinical classification system for thalassemia has been adopted by the Thalassemia International Federation in its recent guidelines and categorizes thalassemia into TDT and NTDT categories, which include various genotypes affecting the α - or β -globin genes, and hemoglobinopathies including hemoglobin E. Categorization into either TDT or NTDT involves a thorough clinical evaluation, including clinical symptoms of anemia, severity of anemia, signs of extramedullary hematopoiesis, and transfusion requirements (Figures 7-5 and 7-6).

Thalassemia minor

α -Thalassemia trait

In contrast to β -thalassemia, α -thalassemia can manifest in both fetal and postnatal life. The clinical manifestations of α -thalassemia are related to the number of functional α -globin genes (Figure 7-4). Heterozygotes for α^+ -thalassemia ($-\alpha/\alpha\alpha$), so-called silent carriers, are clinically normal with minimal to no anemia, or morphologic abnormalities of RBCs. The hemoglobin electrophoresis is normal. Thalassemia trait (2-gene deletion α -thalassemia) occurs in 2 forms: α^0 -thalassemia trait ($--/\alpha\alpha$) or homozygosity for α^+ -thalassemia ($-\alpha/-\alpha$). Individuals with thalassemia trait have a lifelong mild microcytic anemia. In newborns who are heterozygous for α^0 -thalassemia ($--/\alpha\alpha$), hemoglobin electrophoresis reveals 2% to 5% Hb Bart's and microcytosis (MCV < 95 fL). Children and adults heterozygous for α^0 -thalassemia ($--/\alpha\alpha$) or homozygous for α^+ -thalassemia ($-\alpha/-\alpha$) have mild anemia with hypochromic, microcytic RBCs and target cells. The

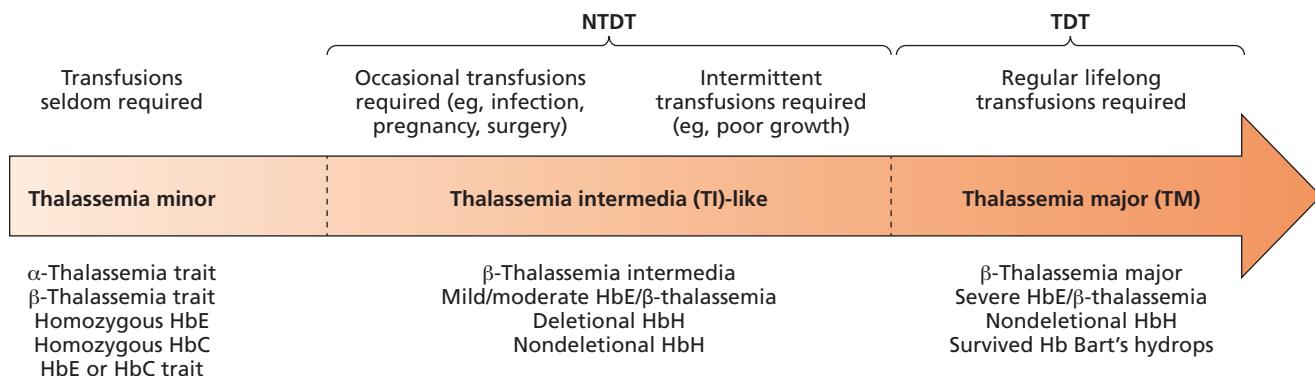


Figure 7-5 The clinical spectrum of thalassemia syndromes based on transfusion requirement. Adapted with permission from Taher A et al, in Weatherall D, ed. Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT). Nicosia, Cyprus: Thalassaemia International Federation; 2013.

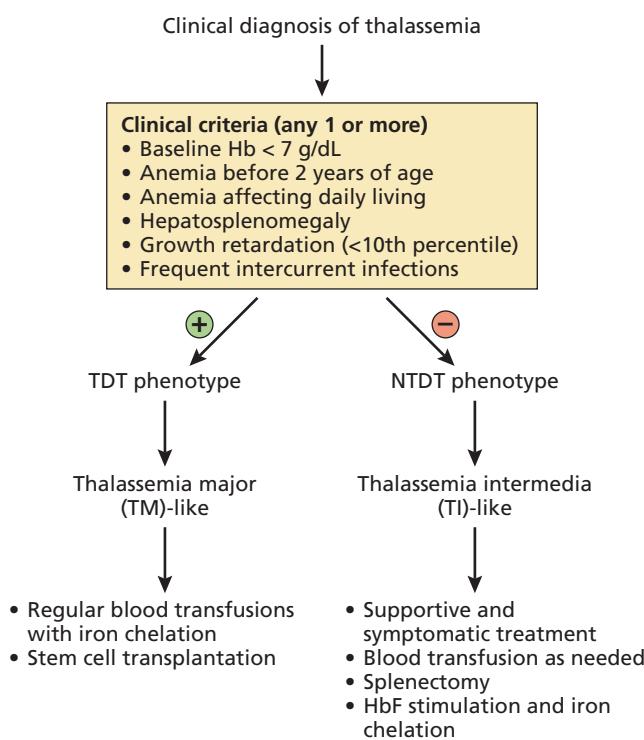


Figure 7-6 Diagnosis, classification and management of TDT and NTDT. Modified from Viprakasit V, Ekwattanakit S, *Hematol Oncol Clin N Am*. 2018;32(2):193–211.

RBC indices are similar to those of β -thalassemia trait, but the hemoglobin electrophoresis is normal ($\text{HbA}_2 < 3.5\%$). Molecular testing is required to confirm the diagnosis in α -thalassemia due to 1 or 2 gene deletions. The high prevalence of the $-\alpha/-\alpha$ genotype in African Americans is noteworthy. About 2% to 3% of all African Americans in the United States have asymptomatic microcytosis and borderline anemia (often mistaken for iron deficiency) as

a result of this condition. Individuals with 1 or 2 alpha gene deletions ($-\alpha/\alpha\alpha, --\alpha\alpha, -\alpha/-\alpha$) do not generally require any specific treatment. Individuals of at-risk ethnicities of childbearing age who are at risk of HbH disease or hydrops fetalis should be offered genetic counseling for informed reproductive choices.

β -Thalassemia trait

β -Thalassemia trait (minor) is asymptomatic and is characterized by mild microcytic anemia. Most commonly, it arises from heterozygous β -thalassemia (β -thalassemia trait). Neonates with β -thalassemia trait have no anemia or microcytosis; these develop with increasing age as the transition from HbF to HbA production progresses. Patients with β -thalassemia trait may have a hemoglobin ranging from 9 g/dL to a normal value. Peripheral smear shows microcytic, hypochromic RBCs, poikilocytes, and target cells. Basophilic stippling is variable. The MCV is usually $<70 \text{ fL}$, the MCH is reduced ($\text{MCH} < 26 \text{ pg}$), and the reticulocyte count can be mildly elevated. HbA_2 levels are diagnostically elevated to $> 3.5\%$ (usually 4% to 7%), and HbF levels may be mildly increased. RBC survival is normal, with minimal ineffective erythropoiesis. Individuals with β -thalassemia trait are asymptomatic and do not require therapy. They should be identified to reduce the risk of inappropriate iron supplementation. Individuals of childbearing age should be offered genetic counseling for informed reproductive choices.

Transfusion-dependent thalassemia

Patients with TDT require regular blood transfusions for survival and include β TM (homozygous β^0 -thalassemia), severe HbE/ β -thalassemia, severe nondeletional HbH disease, and those who survived Hb Bart's hydrops fetalis.

β -Thalassemia major (Cooley's anemia) is characterized by absence of or severe deficiency in β -chain synthesis. Symptoms are usually evident within the first 6 to 12 months of life as the HbF level begins to decline and severe anemia occurs with Hb <7 g/dL. In the absence of adequate RBC transfusions, the infant presents with pallor, irritability, jaundice, failure to thrive and a variety of clinical findings. Erythroid expansion leads to facial deformities, including frontal bossing and maxillary prominence. Increased erythroid expansion widens the bone marrow space, thins out the cortex, and causes low bone density, which may predispose some patients with TDT to fractures. Growth retardation, progressive hepatosplenomegaly, gallstone formation, and cardiac disease are common. Most homozygotes do not survive without transfusions beyond the age of 5 years. RBC transfusions ameliorate severe anemia and suppress ineffective erythropoiesis.

A child with β -thalassemia major who is not receiving transfusions suffers from severe anemia. Peripheral blood smear findings include anisopoikilocytosis, target cells, severe hypochromia, nucleated red blood cells, and basophilic stippling. The reticulocyte count is slightly increased, and nucleated RBCs are abundant. These findings are exaggerated after splenectomy. Hemoglobin electrophoresis reveals persistent elevation of HbF ($\alpha_2\gamma_2$) and variable elevation of HbA₂ ($\alpha_2\delta_2$). HbA is absent in homozygous β^0 -thalassemia.

RBC transfusion has been the mainstay in the management of β -thalassemia major and its complications. The goals of transfusions are to promote normal growth and development and to suppress ineffective erythropoiesis. A lifelong chronic blood transfusion program to maintain a pretransfusion Hb level between 9 and 10 g/dL sufficiently suppresses bone marrow expansion while minimizing transfusional iron loading. An increased incidence of cerebral thrombosis, venous thromboembolism, and pulmonary hypertension has been reported in β -thalassemia major and β -thalassemia intermedia following splenectomy, and these risks should be considered before splenectomy. Often, increasing the transfusion targets is sufficient to reduce the degree of splenomegaly.

Homozygous α^0 -thalassemia (—/—) results in the Hb Barts hydrops fetalis syndrome. The lack of HbF due to the absence of α chains produces intrauterine hypoxia, resulting in marked expansion of bone marrow and hepatosplenomegaly in the fetus and enlargement of the placenta. In utero death usually occurs between 30 and 40 weeks or soon after birth. The blood smear in Hb Barts hydrops fetalis syndrome (—/—) reveals markedly abnormal RBC morphology with anisopoikilocytosis, hypochromia, targets, basophilic stippling, and nucleated RBCs. The hemoglobin electrophoresis in a neonate reveals approximately 80%

Hb Barts and the remainder Hb Portland ($\zeta_2\gamma_2$). A fetus with homozygous α^0 -thalassemia can be rescued with intrauterine transfusions, typically initiated at 24 weeks gestation and continued until term, with fetal middle cerebral artery Doppler velocity monitoring as a guide for the degree of fetal anemia. Such patients need postnatal chronic transfusions throughout life or stem cell transplantation. Maternal complications due to a homozygous α^0 -thalassemia fetus include preeclampsia, hypertension, hemorrhage, dystocia, and retained placenta. Because of the high prevalence of the α^0 -genotype in Southeast Asian and certain Mediterranean populations, screening programs and genetic counseling can reduce the occurrence of births resulting in Hb Barts hydrops fetalis and HbH disease.

Non-transfusion-dependent thalassemia

NTDT includes a wide spectrum of clinical phenotypes, ranging from mild to moderately severe anemia. Patients with NTDT do not require regular blood transfusions for survival. Intermittent transfusions may be required in acutely worsening anemia due to infection or acute illness, or to allow for normal growth and development in childhood. Some patients with NTDT may require regular transfusions later on in life, often in adulthood, due to complications of the disease including worsening anemia and splenomegaly. NTDT encompasses 3 clinically distinct forms of thalassemia, including β -thalassemia intermedia, hemoglobin E/ β -thalassemia, and hemoglobin H disease.

These patients exhibit a wide spectrum of clinical findings, from mild to more significant complications including hepatosplenomegaly, extramedullary hematopoietic pseudotumors, bone deformities, leg ulcers, delayed puberty, thrombotic events, pulmonary hypertension, silent infarcts, gallstones, and iron overload. These complications, except for iron overload, are generally limited in the well-transfused thalassemia patient because transfusion interrupts the underlying pathophysiology. Most indications for initiating a chronic transfusion program in NTDT are similar to those in TDT. However, these are generally initiated later in childhood or in adulthood, depending on the severity of the phenotype. Some patients may present with mild phenotypes in childhood, and subsequently develop worsening anemia, increased extramedullary hematopoiesis, and endocrine complications in adulthood that may warrant initiation of a chronic transfusion program. Thus it is important to closely follow all individuals with NTDT long term, with regular interval evaluation of complications (Table 7-2).

A variable degree of anemia with hypochromic, microcytic cells and target cells is observed in NTDT. Laboratory abnormalities are similar to β -thalassemia trait, but more severe.

The clinical manifestations are variable in HbH disease ($-/-\alpha$), with severe forms resulting in transfusion dependence, and other individuals having a milder course. As in β -thalassemia intermedia, splenomegaly occurs commonly in the anemic patient. The homozygous state for Hb Constant Spring results in moderate anemia with splenomegaly. HbH disease ($-/-\alpha$) is characterized by anisopoikilocytosis and hypochromia with elevated reticulocyte counts. Hemoglobin electrophoresis reveals 5% to 40% of the rapidly migrating HbH. Supravital staining with brilliant cresyl blue reveals inclusions representing in vitro precipitation of HbH. Patients with HbH disease are categorized as NTDT and usually require no specific interventions. However, nondeletional hemoglobin H disease, such as HbH Constant Spring ($-/\alpha^{\text{CS}}\alpha$) is typically more severe than classical HbH disease ($-/-\alpha$) and individuals often require intermittent or chronic RBC transfusions. Since the forms of thalassemia that start as NTDT at a young age may have a variable phenotype with increasing age, close observation and follow up is important.

Clinical complications

Complications in TDT and NTDT affect multiple systems and are due to chronic hemolysis, ineffective erythropoiesis, increased intestinal iron absorption, and transfusional iron overload. Management of patients with TDT and NTDT

involves a comprehensive multidisciplinary care approach. Table 7-1 summarizes the difference in complications between TDT and NTDT.

Iron overload

Iron overload is a major complication in TDT and NTDT. Each milliliter of transfused blood contains 1 mg of iron. Red cell transfusions are the major cause of iron loading in TDT. Iron accumulates because the body does not have an active mechanism to excrete excess iron. Excess iron results in increased nontransferrin-bound iron, which generates harmful reactive oxygen species leading to lipid peroxidation, and organelle and DNA damage causing apoptosis, fibrosis, and organ damage. Uncontrolled transfusional iron loading leads to iron deposition in key organs leading to an increased risk of liver cirrhosis, hepatocellular carcinoma, heart failure, and endocrine complications including hypogonadotropic hypogonadism, diabetes, hypothyroidism, osteoporosis, and hypoparathyroidism. An increased frequency of *Yersinia enterocolitica* bacteremia is associated with iron overload and chelation therapy with deferoxamine. Over the last few years, patient survival has significantly improved due to improved iron chelation therapy, improved modalities to measure liver and cardiac iron load, and a comprehensive care approach. Adherence to chelation is essential for improved clinical outcomes.

Table 7-1 Complications in TDT and NTDT

Complication	TDT	NTDT	Management	Monitoring
Heart failure	+++	+	Iron chelation, standard cardiac care	Cardiac MRI Echocardiogram EKG
Arrhythmia	+	++	Standard care	EKG
Viral hepatitis	+++	+	Hepatitis B vaccination, antiviral therapy	Viral serologies
Hepatic fibrosis, cirrhosis, and hepatocellular carcinoma	++	+++	Standard care	Liver MRI, FibroScan, ultrasound
Growth retardation	++	+	Transfusion, chelation, and hormonal therapy	Clinical growth evaluation
Delayed puberty	++	+	Transfusion, chelation, and hormonal therapy	Tanner stage
Glucose intolerance/diabetes	++	+	Chelation and standard care	Lab monitoring
Decreased bone mineral density	++	+++	Standard and specific therapy	Bone densitometry
Extramedullary masses	+	+++	Hypertransfusions, hydroxyurea or radiation	Clinical history, CT scan, MRI
Thrombosis	+	+++	Anticoagulation, transfusion	
Pulmonary hypertension	+	+++	Standard care, sildenafil, bosentan	Echocardiogram
Leg ulcers	+	++	Topical treatment, hydroxyurea	

Adapted from Marcon A et al, *Hematol Oncol Clin N Am*. 2018;32:223–236.

Refer to Cappellini MD et al, eds. Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT). 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation; 2014 and Taher A et al, eds. Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT). Nicosia, Cyprus: Thalassaemia International Federation; 2013 for general guidelines on clinical and laboratory evaluation for complications of thalassemia.

CT, computed tomography; EKG, electrocardiogram; MRI, magnetic resonance imaging; NTDT, non-transfusion-dependent thalassemia; TDT, transfusion-dependent thalassemia.

Monitoring iron load is key to establishing an individualized, effective iron chelation regimen for each patient. Iron load is determined by serum ferritin, liver iron concentration, and cardiac iron load. Serum ferritin moderately correlates with body iron stores and is an easy, convenient, and inexpensive measure to trend. However, it has several limitations since it is an indirect measure of true body iron burden, is an acute phase reactant, and has a nonlinear response to iron load at high levels. Liver iron concentration (LIC) can be determined by liver biopsy or by the new gold standard, liver MRI (magnetic resonance imaging) R2. Normal LIC is <1.8 mg Fe/g dry weight. Cardiac MRI T2^{*} correlates with cardiac iron load and the risk of developing heart failure increases with T2^{*} values <20 ms. The risk for developing heart failure is highest when the cardiac T2^{*} is <8 ms. A complete iron load evaluation includes at least serum ferritin every 3 months, yearly LIC by MRI R2 starting at age 5, and yearly cardiac iron T2^{*} starting at 8 to 10 years of age. For young children, the risks of sedation should be weighed against the risks of severe liver iron overload. The main goals of iron chelation therapy are to maintain safe levels of body iron to prevent iron overload and its complications and to reduce accumulated iron. Iron chelation therapy is tailored to each individual based on transfusion rates and iron burden. In TDT, iron chelation therapy with subcutaneous deferoxamine or oral deferasirox is initiated when serum ferritin levels reach approximately 1,000 to 1,500 ng/mL following approximately 12 months of scheduled transfusions or approximately 20 units of blood. Chelation is adjusted to

maintain a ferritin of <1,000 ng/mL, an LIC of <5 mg of iron/g dry weight, and a cardiac T2^{*} of >20 ms. In those with significant cardiac iron burden, combination therapy including deferiprone can reduce cardiac iron. Monitoring for chelator-specific complications should be performed. The different chelators and their properties are summarized in Table 7-2.

Iron overload in NTDT occurs primarily due to increased gastrointestinal absorption in the setting of ineffective erythropoiesis. Thus, even in the absence of transfusion, some patients may develop iron overload, which significantly increases with increasing number of transfusions. Iron-associated complications are similar to those seen in TDT, except cardiac siderosis is much less common. Serum ferritin and LIC measurements show a moderately positive correlation and should be regularly evaluated in all patients over 10 years of age. In NTDT, the total body iron load may be higher than what the serum ferritin levels suggest. Thus a serum ferritin of >800 ng/mL warrants LIC evaluation. Chelation therapy to reduce iron-associated morbidity should be initiated if the LIC is ≥5 mg Fe/g dry weight. Deferasirox has been well studied in NTDT with a good efficacy and safety profile.

Cardiac disease

Cardiovascular complications are the main cause of death in TDT patients. Transfusional cardiac iron overload leads to left ventricular dysfunction and arrhythmias. Patients with significant myocardial iron can be asymptomatic with normal ventricular function for years before developing

Table 7-2 Properties of iron chelators

	Deferoxamine (DFO)	Deferiprone (DFP)	Deferasirox (DFX)	
			Tablet for oral suspension	Film-coated tablet
Route	SC or IV infusion	Oral (tablet or syrup)	Oral	
Dose	20–60 mg/h over 8–24 h	75–100 mg/kg/d	20–40 mg/kg/d	14–28 mg/kg/d
Schedule	5–7 times a week	3 times daily	Once daily	
Excretion	Urine, feces	Urine	Feces	
Remove liver iron	+++	++	+++	
Remove cardiac iron	++	+++	++	
Side effects	Injection site reaction Allergy High-frequency hearing loss Retinopathy Poor growth <i>Yersinia</i> infections	GI (nausea, vomiting, abdominal pain) Increased ALT Arthralgias Neutropenia Agranulocytosis (requires weekly monitoring)	GI (diarrhea, vomiting, nausea, abdominal pain) Skin rash Increased ALT Increased serum creatinine GI bleeding	

ALT, alanine aminotransferase; GI, gastrointestinal.

heart failure. It is thus important to specifically monitor for cardiac iron load with cardiac MRI T2*. Prevention of cardiac iron overload with chelation adherence must be emphasized. Once cardiac iron overload develops, intensive chelation with monotherapy or combination therapy is key. Cardiac iron overload with heart failure can be successfully reversed with aggressive chelation therapy. Close follow-up with a cardiologist is important because many of these patients also benefit from other cardiac medications.

Pulmonary hypertension is the major cardiovascular complication in NTDT patients. The pathophysiology is multifactorial due to endothelial dysfunction, hypercoagulability, increased vascular tones, inflammation, nitric oxide depletion due to hemolysis, and splenectomy. Regularly transfused TDT patients have lower prevalence of pulmonary hypertension, suggesting a therapeutic role for transfusion therapy in NTDT patients who develop this complication. Sildenafil citrate, bosentan and epoprostenol have been shown to be beneficial in the thalassemia population with pulmonary hypertension.

Liver disease

Many adults with TDT have chronic HCV infection resulting from contaminated RBC products that they received before the early 1990s. The concomitant presence of both HCV infection and iron overload significantly increases the risk of hepatic fibrosis. Treatment with ribavirin-based regimens may be complicated by hemolysis resulting from ribavirin and has been limiting in thalassemia patients. New nonribavirin treatment regimens with direct-acting antivirals have shown sustained viral response rates in thalassemia (97.6%) similar to that in patients without hemoglobinopathies. Thalassemia patients are at risk for hepatocellular carcinoma, especially those with histories of untreated liver iron overload and concurrent HCV infection.

Endocrine complications

Endocrine complications are very common in thalassemia patients, primarily due to effects of iron deposition in the anterior pituitary or endocrine organs beginning in childhood. Endocrinopathies are generally more common in patients with TDT compared to NTDT due to the significantly increased transfusional iron burden. All TDT and NTDT patients should be routinely followed by an endocrinologist with regular monitoring for endocrinopathies.

Hypogonadotropic hypogonadism (secondary hypogonadism) is the most common endocrinopathy in patients with thalassemia, occurring in 50% to 90% of patients. In children it can present as delayed puberty, while in adults decreased libido, infertility, and osteoporosis are common.

NTDT patients generally have normal puberty and are fertile due to less iron burden. Appropriate chelation with good adherence is important in preventing hypogonadism.

Diabetes is common in 20% to 30% of adult patients with TDT and strongly correlates with severity of iron overload, inadequate chelation, poor adherence, and late initiation of chelation therapy. Hemoglobin A1c is an unreliable marker of glycemic control in thalassemia patients due to changes in hemoglobin balance and frequent transfusions. Fructosamine is a more reliable marker of diabetic control and can be used to follow diabetic treatment and control which is similar to the general population. Fructosamine is indicative of glycemic control over the past 3 weeks.

Hypothyroidism occurs in about 10% of patients with TDT and strongly correlates with the severity of anemia and iron overload. Well-treated patients with TDT are unlikely to develop hypothyroidism. In those with subclinical hypothyroidism, intensification of chelation therapy can help improve thyroid function. Hypoparathyroidism and adrenal insufficiency are less commonly seen in thalassemia patients and are due primarily to iron overload.

Bone disease

Low bone mass and osteoporosis increase the risk of fracture, and are common in TDT and NTDT patients, occurring in up to 90% of individuals. It tends to be more common in NTDT patients. Contributors to decreased bone mineral density in thalassemia include iron overload with direct iron toxicity on osteoblasts, ineffective erythropoiesis, hypogonadism, iron chelation with deferoxamine, vitamin D deficiency, hypercalcioria, and decreased weight-bearing exercises. Diagnosis involves yearly bone densitometry starting at 10 years of age. Treatment involves a multifaceted approach including optimizing transfusions, chelation therapy, treatment of concurrent endocrinopathies, vitamin D replacement, bisphosphonate therapy, physical activity, and smoking cessation. Bisphosphonates have been shown to reduce bone resorption, increase bone mineral density, reduce back pain, and improve quality of life in thalassemia patients.

Other complications

Thalassemia is considered a hypercoagulable state, especially in NTDT where the incidence is as high as 20%. Splenectomy further increases the risk of thrombosis. Overt stroke and silent cerebral infarcts are also increased in thalassemia, especially NTDT. Increased ineffective erythropoiesis in poorly controlled thalassemia results in expansion of extramedullary masses beyond the liver and spleen. Paraspinal masses can cause spinal cord compression. Management involves hypertransfusion, hydroxurea, and in urgent situations, radiation therapy. Leg ulcers are more commonly seen

in NTDT due to reduced tissue oxygenation and increase with increasing age and iron burden.

Curative options in thalassemia

Allogeneic bone marrow transplantation from a histocompatible (human leukocyte antigen [HLA]-compatible) sibling has been performed in >1,000 thalassemia major patients and is curative in most. The outcome is influenced by the age of the patient and disease status at the time of transplant. The Pesaro prognostic score helps predict transplant outcomes in patients younger than 17 years old. The 3 key prognostic factors, which are indirect estimates of the disease burden and degree of iron overload, include (i) hepatomegaly > 2 cm, (ii) portal fibrosis, and (iii) history of inadequate iron chelation therapy. Over the years, improvements in conditioning regimens, prevention and management of graft-vs-host disease, improved techniques for HLA-typing, and overall supportive care, have significantly improved overall survival to over 90% and thalassemia-free survival to over 80%. Recent studies exploring unrelated donor transplantation, haploidentical transplantation, and nonmyeloablative regimens are encouraging, even in patients with prior iron loading or concomitant HCV infection.

Since allogeneic stem cell transplantation is not available to most patients with thalassemia due to the lack of matched donors, globin gene therapy offers a promising new curative approach. Preliminary results in gene therapy for TDT have led to transfusion independence in some subjects and are promising for the future. Work continues to determine the optimal factors that influence gene therapy outcomes including patient factors, vector properties, transduction efficiency, and conditioning regimens.

CLINICAL CASE (continued)

The patient presented in this case likely has 2 copies of alpha deletions in the trans position ($-{\alpha}-\alpha$) because she is of African descent. Patients with this condition usually have mild microcytic, hypochromic anemia. Targeted RBC forms suggest the presence of thalassemia in an otherwise healthy person. With single or double α -gene deletions, the hemoglobin electrophoresis is typically normal, unlike in β -thalassemia. α -Thalassemia is often a diagnosis of exclusion, and identification of similar findings in family members supports the diagnosis. Molecular testing for specific α -gene deletions confirms the diagnosis. Iron deficiency should be ruled out. Exogenous iron should not be prescribed because it is unnecessary and potentially harmful. Patients are generally asymptomatic, require no treatment, and have a normal life expectancy.

KEY POINTS

- The thalassemias are characterized by a reduced rate of synthesis of one of the globin subunits of the hemoglobin molecule.
- The intracellular precipitation of the excess, unpaired globin chains in thalassemia damages red cell precursors and circulating red cells, resulting in ineffective erythropoiesis and hemolysis.
- The α -thalassemias are primarily due to DNA deletions. Four α -genes are normally present, so multiple phenotypes are possible when gene deletions occur.
- The β -thalassemias are caused by >200 different mutations, usually point mutations, with a wide variety of genetic abnormalities documented.
- α -thalassemia trait is characterized by mild asymptomatic anemia with microcytic indices and a normal hemoglobin electrophoresis.
- The hemoglobin electrophoresis in β -thalassemia trait reveals increased levels of hemoglobin A₂ and variably increased hemoglobin F.
- Thalassemia can be clinically classified into transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT).
- Patients with TDT require regular blood transfusions for survival, while those with NTDT who have a mild to moderate phenotype require intermittent transfusions during periods of acute illness, infection, or pregnancy, or to allow for normal growth and development.
- Iron overload is a complication of TDT and NTDT, and monitoring of iron load with serum ferritin, and liver and cardiac iron content by MRI are important to optimize chelation therapy initiation and management.
- Hemolytic anemia, ineffective erythropoiesis, and iron overload contribute to multiple complications of TDT and NTDT including bone deformities, cardiac failure, arrhythmia, liver cirrhosis, HCV infection, thrombosis, endocrinopathies, osteoporosis, leg ulcers, and pulmonary hypertension.
- Partner testing and genetic counseling in individuals with α -thalassemia trait is important so that a pregnant woman with a risk of a homozygous α^0 -thalassemia fetus can consider further testing, early termination, or undergo intrauterine transfusions to support fetal growth should they wish to maintain the pregnancy.

Sickle cell disease

CLINICAL CASE

A 17-year-old African American male with homozygous sickle cell anemia (HbSS) is admitted to the hospital with a 4-day history of a typical painful episode involving his arms and legs. There is no recent febrile illness. Past medical history is remarkable for few hospital admissions for pain crises and red cell transfusion once as a young child. He is in severe pain and appears ill, and vital signs are remarkable for a pulse of 129 and temperature of 38.5°C. Scleral icterus and moderate jaundice are noted. Laboratory data include hemoglobin 7.2 g/dL (baseline 9.1 g/dL), corrected reticulocyte count of 2%, and platelet count 72,000/µL. Liver function tests are elevated above baseline and include a direct bilirubin of 4.8 mg/dL, aspartate aminotransferase (AST) of 1,200 U/L, and alanine aminotransferase (ALT) 1,550 U/L. His creatinine is elevated at 4.3 mg/dL. Abdominal ultrasound is nondiagnostic. He is immediately started on intravenous fluids and opioid analgesics. Broad-spectrum antibiotics are empirically administered. Over the next 24 hours he becomes tachypneic and slightly confused. Hypoxemia develops despite oxygen supplementation, and anuria ensues. Serum creatinine has increased to 6.4 mg/dL, direct bilirubin to 7.8 mg/dL, AST to 2,725 U/L, and creatine phosphokinase to 2,200 IU/L and hemoglobin has decreased to 5.8 g/dL. The patient undergoes simple transfusion and subsequently red cell exchange. Acute dialysis is required. He slowly improves during a prolonged 3-week hospitalization. No infectious etiology was identified.

Sickle hemoglobin (HbS) was the first hemoglobin variant discovered. It has been well characterized at the biochemical and molecular level. Heterozygosity for the sickle cell gene (β^S), called sickle cell trait, occurs in >20% of individuals in equatorial Africa; up to 20% of individuals in the eastern provinces of Saudi Arabia and central India; up to 6.3% in Hispanic populations; and approximately 5% of individuals in parts of the Mediterranean region, the Middle East, and North Africa. In HbS, a hydrophobic valine is substituted for the normal, more hydrophilic glutamic acid at the sixth residue of the β -globin chain (Figure 7-7). This substitution is due to a single nucleotide mutation (GAG/GTG) in the sixth codon of the β -globin gene. Heterozygous inheritance of HbS offers a degree of protection from severe malaria infection. This has been offered as an explanation for the evolutionary selection of the HbS gene despite the devastating effects of the homozygous state. The β^S gene is inherited in an autosomal codominant fashion. That is, heterozygous inheritance does not cause disease, but is detectable (sickle cell trait);

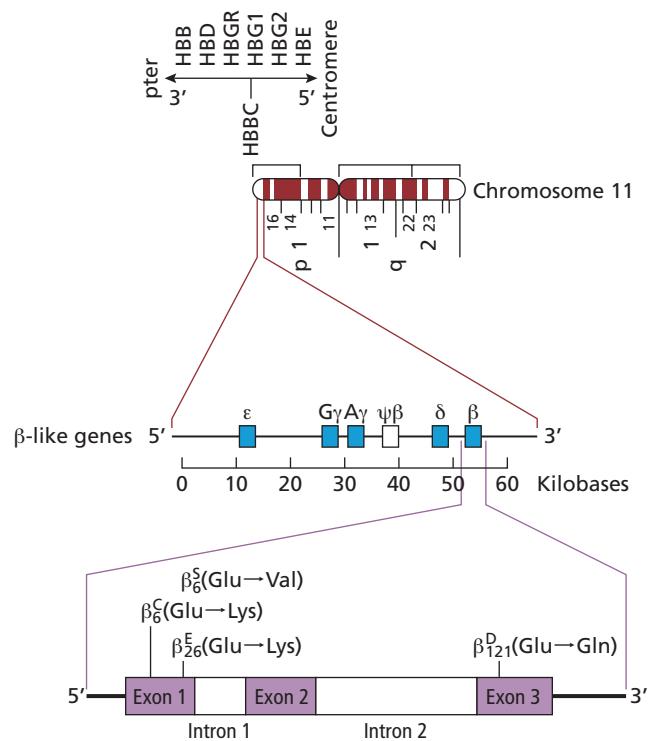


Figure 7-7 Common β -globin variants. The locations of the mutations within the chromosome (top), the β -globin cluster (middle), and the β -globin gene itself (bottom) are shown for 4 common β -globin variants.

homozygous inheritance or compound heterozygous inheritance with another mutant β -globin gene results in disease. The *sickle cell syndromes* include all conditions with E6V mutation, mostly when β^S is inherited (including sickle cell trait). In contrast, the term *sickle cell disease* includes only those genotypes associated with varying degrees of chronic hemolytic anemia and vaso-occlusive pain (not sickle trait): homozygous sickle cell anemia (HbSS), sickle-HbC disease (HbSC), sickle β^0 -thalassemia (HbS β^0), and sickle β^+ -thalassemia (HbS β^+). Less common hemoglobin mutants, such as O^{Arab}, D^{Punjab}, or E, may be inherited in compound heterozygosity with β^S to result in sickle cell disease.

Sickle cell trait (HbAS) occurs in 8% to 9% of the African American population. It is associated with the rare complications of hematuria, renal papillary necrosis, pyelonephritis during pregnancy, and risk of splenic infarction at high altitude. Sickle trait also is associated with the extremely rare medullary carcinoma of the kidney, and an increased risk of sudden death during extreme conditions of dehydration and hyperthermia. Recent publications have shown that individuals with sickle trait are at higher risk of chronic kidney disease and venous thromboembolism.

This simple heterozygous state generally has a hemoglobin A:S ratio of approximately 60:40, because of the greater electrostatic attraction of α -chains to β^A rather than β^S chains. When the availability of α chains is limited by co-inherited α -thalassemia, the A:S ratio is further increased.

Pathophysiology

The hallmark of sickle cell pathophysiology is the intraerythrocytic polymerization of deoxyhemoglobin S. When deoxygenation of HbS occurs, the normal conformational change of the tetramer exposes on its external surface a hydrophobic β_6 valine (instead of the hydrophilic glutamate of HbA), resulting in decreased solubility and a tendency of deoxyhemoglobin S tetramers to aggregate or polymerize. The rate and degree of this polymerization determines the rheologic impairment of sickle erythrocytes and the change in morphology for which the condition was named. Polymerization rate and extent are related to the intracellular concentration of HbS, the type and fractional content of other hemoglobins present (particularly HbF), and percent oxygen saturation. These variables correlate with the rate of hemolysis in sickle cell syndromes.

Multiple factors determine the clinical manifestations of sickle cell disease. In addition to physiologic changes such as tissue oxygenation and pH, multiple genetic polymorphisms and mutations may modify the presentation of the disease. This is best appreciated by examining the influence of the coinheritance of other hemoglobin abnormalities on the effects of HbS. For example, the coexistence of α -thalassemia reduces the hemolytic severity as well as the risk of cerebrovascular accidents. High levels of fetal hemoglobin (HbF) may substantially reduce symptoms as well as clinical consequences. Compound heterozygosity for a second abnormal hemoglobin (eg, HbC, D, or E) or β -thalassemia also modifies some of the manifestations of disease (discussed later in this section) (Table 7-2).

Several restriction fragment-length polymorphisms (RFLPs) may be identified in the vicinity of a known gene and define the genetic background upon which a disease-causing mutation has arisen. For example, the coinheritance of a defined set of RFLPs around the β -globin gene can define a disease-associated “haplotype” that marks the sickle mutation within a specific population. These β -globin haplotypes have also been associated with variations in disease severity. This association is probably not related to the RFLPs themselves, but rather is mediated through linked differences in γ -chain (HbF) production. The β^S gene has been found to be associated with 5 distinct haplotypes, referred to as the Benin (Ben), Senegal (Sen), Central African Republic (CAR or Bantu), Cameroon (Cam), and Arab-Indian (Asian) haplotypes. This is

evidence that the β^S gene arose by 5 separate mutational events. In general, the Asian and Sen haplotypes are associated with a milder clinical course, and CAR is associated with a more severe course.

Although the deoxygenation-polymerization-sickling axiom provides a basic understanding of sickle cell disease, there is an increasing appreciation that interactions of sickle cells with other cells and proteins contribute to the hemolytic and vaso-occlusive processes. The chronic hemolytic nature of sickle cell disease leads to chronic depletion of nitric oxide both from the release of arginase and also free heme. Free heme is associated with impaired cleavage of large von Willebrand factor multimers by ADAMTS 13 and also with the activation of toll-like receptor 4 (TLR4). Heme-induced TLR4 has been shown to cause both endothelial activation and vaso-occlusion in murine models of sickle cell disease. Additionally, infusion of hemein, the oxidized prosthetic moiety of hemoglobin, in a murine model has been associated with acute intravascular hemolysis and the rapid development of acute chest syndrome. Inhibition of TLR4, along with hemopexin infusions, has prevented mice from developing acute chest syndrome. Indeed, the relationship between free heme and TLR4 appears to be a vital component in the development of vaso-occlusion and acute chest syndrome in sickle cell disease. In vitro data show that sickle erythrocytes exhibit abnormally increased adherence to vascular endothelial cells, as well as to subendothelial extracellular matrix proteins. Apparent endothelial damage is demonstrated by the increased number of circulating endothelial cells in sickle cell disease patients, and by the increase in such cells during vaso-occlusive crises. The disruption of normal endothelium results in the exposure of extracellular matrix components, including thrombospondin, laminin, and fibronectin. Endothelial cell receptors include the vitronectin receptor $\alpha_v\beta_3$ integrin and the cytokine-induced vascular cell adherence molecule-1. RBC receptors include CD36 (glycoprotein IV), the $\alpha_{IV}\beta_1$ integrin, the Lutheran blood group glycoproteins, and sulfatides. Vaso-occlusion thus may be initiated by adherence of sickle erythrocytes to endothelial cells and extracellular matrix molecules exposed during the process of endothelial damage and completed by trapping of sickled, nondeformable cells behind this nidus of occlusion. Activation of blood coagulation, resulting in enhanced thrombin generation and evidence for platelet hyperreactivity, has been demonstrated in patients with sickle cell disease during steady-state and vaso-occlusive episodes. It has been suggested that the exposure of RBC membrane phosphatidylserine and circulating activated endothelial cells in sickle cell disease patients contribute to the hypercoagulability by providing proco-

agulant surfaces. The correlation of elevated white blood cell counts to increased mortality and adverse outcomes identified by epidemiologic studies of sickle cell disease patients suggest that neutrophils also participate in vaso-occlusion. This concept has been further supported by the precipitation of vaso-occlusive episodes with markedly increased neutrophil counts associated with the administration of granulocyte colony-stimulating factor. These findings taken together support the concept that the products of multiple genes, as well as inflammatory cytokines, contribute to the pathology of sickle cell disease.

Laboratory features

The diagnosis of the sickle cell syndromes is made by the identification of HbS in erythrocyte hemolysates. Historically, cellulose acetate electrophoresis at alkaline pH was used to separate HbA, HbA₂, and HbS; and citrate agar electrophoresis at acidic pH was used to separate co-migrating HbD and HbC from HbS and HbA₂, respectively. Currently, high-performance liquid chromatography (HPLC) and isoelectric focusing are used in most diagnostic laboratories to separate Hbs. In both HbSS and S β^0 -thalassemia, no HbA is present. In HbSS, however, the MCV is normal, whereas in HbS β^0 -thalassemia, the MCV is reduced. HbA₂ is elevated in S β^0 -thalassemia, but it also can be nonspecifically elevated in the presence of HbS, so an elevation of A₂ alone cannot reliably distinguish HbSS from S β^0 -thalassemia. In sickle cell trait and S β^+ -thalassemia, both HbS and HbA are identified. The A:S ratio is 60:40 in sickle trait (more A than S) and approximately 15:85 in S β^+ -thalassemia (more S than A). Microcytosis, target cells, anemia, and clinical symptoms occur only in S β^+ -thalassemia and not in sickle trait (Table 7-2). Review of the peripheral smear reveals the presence of irreversibly sickled cells in HbSS and HbS β^0 -thalassemia (Figure 7-8), but only rarely in HbS β^+ -thalassemia and HbSC. Turbidity tests (for HbS) are positive in all sickle cell syndromes, including HbAS (sickle trait). The classic sickle cell slide test or “sickle cell prep” (using sodium metabisulfite or dithionite) and the turbidity test detect only the presence of HbS, so they do not differentiate sickle cell disease from sickle cell trait. Therefore, they have limited utility. Sickle cell disease can be diagnosed by DNA testing of the preimplanted zygote in the first trimester of pregnancy using chorionic villus sampling, in the second trimester using amniocentesis, or after birth using peripheral blood.

Clinical manifestations

Two major physiologic processes—shortened RBC survival (hemolysis) and vaso-occlusion—account for most of the

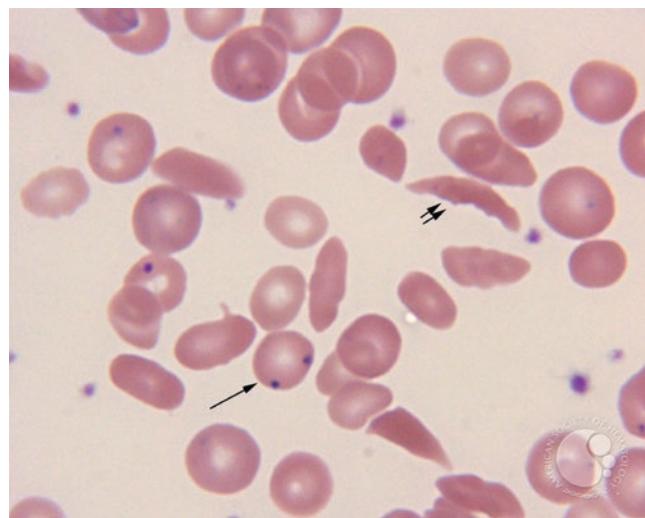


Figure 7-8 Irreversibly sickled cell. This peripheral blood film shows an irreversibly sickled cell (ISC) that occurs in sickle cell anemia (SS), S β^0 -thalassemia (double arrow). ISCs are rare in hemoglobin SC and S β^+ -thalassemia. Also note the Howell-Jolly bodies in this view (single arrow). Source: ASH Image Bank/John Lazarchick (image 00003961).

clinical manifestations of sickle cell disease. The erythrocyte lifespan is shortened from the normal 120 days to approximately 10 to 25 days, resulting in moderate to severe hemolytic anemia, with a steady-state mean hemoglobin concentration of 8 g/dL (ranging from 6 to 9 g/dL) in HbSS disease. The anemia is generally well tolerated because of compensatory cardiovascular changes and increased levels of 2,3-BPG. Several conditions are associated with acute or chronic declines in the hemoglobin concentration, which may lead to symptomatic anemia (Table 7-3). The transient aplastic crisis resulting from erythroid aplasia is caused by human parvovirus infection, which may result in severe or life-threatening anemia. Lesser degrees of bone marrow “suppression” are associated with other infections. Sudden anemia may be caused by acute splenic sequestration in children with HbSS or S β^0 (and in adults with HbSC or S β^+ -thalassemia) or, less frequently, hepatic sequestration, concomitant glucose-6-phosphate dehydrogenase (G6PD) deficiency, or superimposed autoimmune hemolysis. Chronic exacerbations of anemia may be the result of folate or iron deficiency or reduced erythropoietin levels due to chronic renal insufficiency. Because of the chronic erythrocyte destruction, patients with sickle cell disease have a high incidence of pigmented gallstones, which are often asymptomatic.

The acute painful “vaso-occlusive crisis” is the stereotypical and most common complication of sickle cell disease. These often unpredictable events are thought to be caused by obstruction of the microcirculation by

Table 7-3 Typical clinical and laboratory findings of the common forms of sickle cell disease after 5 years of age

Disease	Clinical severity	S (%)	F (%)	A ₂ (%)*	A (%)	Hemoglobin (g/dL)	MCV (fL)
SS	Usually marked	>90	<10	<3.5	0	6–9	>80
S β^0	Marked to moderate	>80	<20	>3.5	0	6–9	<70
S β^+	Mild to moderate	>60	<20	>3.5	10–30	9–12	<75
SC	Mild to moderate	50	<5	0†	0	10–15	75–85
S ⁻ HPFH	Asymptomatic	<70	>30	<2.5	0	12–14	<80

*HbA₂ can be increased in the presence of HbS, even in the absence of β -thalassemia. The classical findings are shown here.

†50% of hemoglobin C migrates near hemoglobin A₂ on alkaline gel electrophoresis or isoelectric focusing.

HPFH, hereditary persistence of fetal hemoglobin; MCV, mean corpuscular volume.

erythrocytes and other blood cells, leading to painful tissue hypoxia and infarction. They most commonly affect the long bones, back, chest, and abdomen. Acute pain events may be precipitated by dehydration, cold temperatures, exercise (in particular swimming), pregnancy, infection, or stress. Often no precipitating factor can be identified. Painful episodes may or may not be accompanied by low-grade fever.

One of the first manifestations of sickle cell disease, acute dactylitis (hand-foot syndrome), results from bone marrow necrosis of the hands and feet. The first attack generally occurs between 6 and 18 months of life, when the HbF level declines. Dactylitis is uncommon after age 3 years, as the site of hematopoiesis shifts from the peripheral to the axial skeleton. Long-bone infarcts with pain and swelling may mimic osteomyelitis. Other skeletal complications of sickle cell disease include osteomyelitis, particularly due to *Salmonella* and staphylococci, and avascular necrosis, especially of the femoral and humeral heads.

Sickle cell disease is a multisystem disorder. Organ systems subject to recurrent ischemia, infarction, and chronic dysfunction include the lungs (acute chest syndrome, pulmonary fibrosis, pulmonary hypertension, hypoxemia), central nervous system (overt and covert cerebral infarction, subarachnoid and intracerebral hemorrhage, seizures, cognitive impairment, moyamoya disease, cerebral vasculopathy), cardiovascular system (cardiomegaly, congestive heart failure), genitourinary system (hyposthenuria, hematuria, proteinuria, papillary necrosis, glomerulonephritis, priapism), spleen (splenomegaly, splenic sequestration, splenic infarction and involution, hypersplenism), eyes (retinal artery occlusion, proliferative sickle retinopathy, vitreous hemorrhage, retinal detachment), and skin (leg ulcerations). The risk of life-threatening septicemia and meningitis because of encapsulated organisms, such as *Streptococcus pneumoniae*, is increased markedly in children with sickle cell disease. This susceptibility is related to functional and anatomic asplenia and decreased opsonization because of

deficient production of natural antibodies. The risk for such infections persists into adulthood.

There are many important clinical differences among the genotypes that cause sickle cell disease (Table 7-2). Hemoglobin SS is associated with the most severe anemia, most frequent pain, and shortest life expectancy (median age, 42 years for men and 48 years for women in one large, but old, study), although there is tremendous heterogeneity in these variables even within this genotype. Hemoglobin S β^0 -thalassemia can closely mimic HbSS, despite the smaller red blood cells, lower MCH concentrations, and higher levels of HbF and HbA₂ associated with this genotype. Patients with HbSC generally live longer lives (median age, 60 years for men and 68 years for women) and have less severe anemia (~20% are not anemic at all), higher MCH concentrations and less frequent pain, but they have more frequent ocular and bone complications. Although HbC does not enter into the deoxyhemoglobin S polymer, patients with HbSC have symptoms, whereas those with sickle cell trait (AS) do not. This is thought to be caused by 2 important consequences of the presence of HbC: the HbS content in HbSC is 10% to 15% higher than that seen in sickle trait (HbS of approximately 50% vs 40%), and the absolute intraerythrocytic concentration of total Hb is increased. The latter phenomenon results from persistent loss of cellular K⁺ and water from these cells induced by the toxic effect of HbC on cell membranes. Another effect of this dramatic cellular dehydration is the generation of target cells, which are far more prevalent on the peripheral smear than sickled forms (Figure 7-9). Finally, in HbSC disease, the increased hematocrit combined with the higher MCH concentration (MCHC) and cellular dehydration results in higher whole blood viscosity, which may increase the likelihood of vaso-occlusion. Patients with HbS β^+ -thalassemia have less severe anemia and pain than patients with HbS β^0 -thalassemia. This is the result of smaller cells, lower MCHC, increased content of HbF and HbA₂ and, most important, the presence of signif-

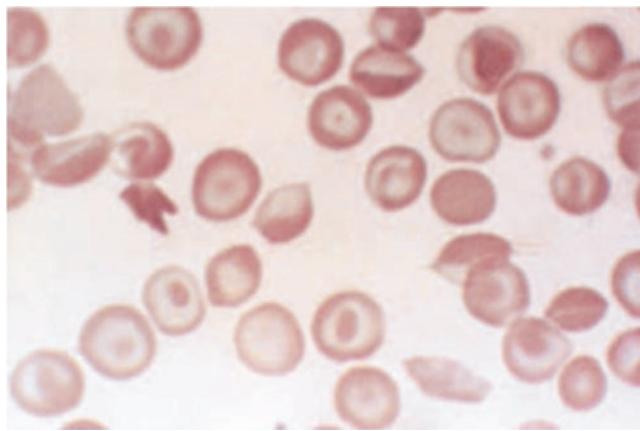


Figure 7-9 Sickle-hemoglobin C disease. This peripheral blood film shows no irreversibly sickled cells, as expected for hemoglobin SC, but shows instead a large number of target cells and several dense, contracted, and folded cells containing aggregated and polymerized hemoglobin.

ificant amounts (10% to 30%) of HbA that interferes with polymerization.

Treatment

Treatment of sickle cell disease includes general preventive and supportive measures, as well as treatment of specific complications. The National Institutes of Health recently published “Evidence-based management of sickle cell disease expert panel report, 2014: guide to recommendations” which is an excellent resource for addressing the spectrum of treatment issues. Table 7-4 summarizes the results of major clinical trials influencing current clinical practice.

Preventive interventions

Children should receive the pneumococcal vaccination, meningococcal vaccination, *Haemophilus influenzae* vaccination, and hepatitis B vaccination (please see adapted table below regarding the most recent guidelines). Additionally, children should have twice-daily penicillin prophylaxis at least until the age of 5 years. Vaccinations against influenza on an annual basis and the pneumococcal vaccine at 5-year intervals (after the childhood PCV-13 and PPV-23 vaccinations) should be administered to all patients. Folic acid supplements are used by some to prevent depletion of folate stores and megaloblastic anemia related to chronic hemolysis, but this is probably unnecessary in industrial countries where diets are better and flour is fortified with folate. For patients on chronic transfusion therapy, consider monitoring for iron overload and annual hepatitis and HIV screening. Screening transcranial Doppler (TCD) ultrasonography to determine risk of overt stroke should be performed at least yearly for children of age 2–16 years

Table 7-4 Causes of acute exacerbations of anemia in sickle cell disease

Cause	Comment
Aplastic crisis	Caused by human parvovirus; does not recur
Acute splenic sequestration crisis	Often recurrent in childhood before splenic involution
Acute chest syndrome	Anemia may precede the onset of respiratory signs and symptoms
Vaso-occlusive crisis	Minimal decline only
Hypoplastic crisis	Mild decline; accompanies many infections
Accelerated hemolysis	Infrequent; accompanies infection of concomitant G6PD deficiency
Hepatic sequestration	Rare
Folate deficiency (megaloblastic crisis)	Rare, even in the absence of folate supplementation

G6PD, glucose-6-phosphate dehydrogenase.

with HbSS or S β^0 -thalassemia (see further discussion of TCD in the sections “Central nervous system disease” and “RBC transfusion” in this chapter). Ophthalmologic examinations should be performed periodically beginning around age 10 years. Genetic counseling services by trained individuals should be available for families with members having sickle cell syndromes.

Painful episodes

Acute pain unresponsive to rest, hydration, and oral analgesics at home requires prompt attention and is the leading cause of hospitalization. Painful episodes can be associated with serious complications, and a high frequency of pain is a poor prognostic factor for survival. It is essential to consider infectious and other etiologies of pain in the febrile patient. A complete blood count should be obtained. Because some degree of negative fluid balance often is present, oral or intravenous hydration is important. Caution must be used with intravenous hydration of adults, especially, who may have decreased cardiac reserve. Prompt administration of analgesics is a priority, and the selection of agents should be individualized based on previous experience. Parenteral opioids, preferably morphine or hydromorphone, are often necessary for both children and adults. The addition of nonsteroidal anti-inflammatory drugs, such as ibuprofen or ketorolac, may decrease the requirement for opioid analgesics but should be used with appropriate vigilance in sickle cell disease because of potential nephrotoxicity. Maintenance analgesia can be achieved with patient-controlled analgesia pumps or with administration at fixed intervals. Constant infusion

Table 7-5 Important completed randomized clinical trials in sickle cell disease

Clinical trial	Year	Outcome
Penicillin Prophylaxis in Sickle Cell Disease (PROPS)	1986	Oral penicillin greatly reduces the incidence of invasive pneumococcal infections in children.
Penicillin Prophylaxis in Sickle Cell Disease II (PROPS II)	1995	Penicillin prophylaxis can be discontinued at 5 years of age.
Multicenter Study of Hydroxyurea in Patients with Sickle Cell Anemia (MSH)	1995	Hydroxyurea reduces the frequency of painful episodes and appears to reduce the frequency of acute chest syndrome, transfusions, and hospitalizations.
National Preoperative Transfusion Study	1995	Simple transfusion to increase the hemoglobin concentration to 10 g/dL is as effective as exchange transfusion to reduce HbS to <30%.
Stroke Prevention Trial in Sickle Cell Anemia (STOP)	1998	First overt stroke can be prevented with red blood cell transfusions in high-risk children identified by TCD ultrasonography.
Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP 2)	2005	Discontinuation of prophylactic red blood cell transfusions after 30 months results in a high rate of reversion to abnormal TCD velocities and stroke.
Hydroxyurea to Prevent Organ Damage in Very Young Children with Sickle Cell Anemia (BABY HUG)	2011	Hydroxyurea starting at 9 to 18 months of age did not prevent splenic and renal damage (the trial's primary endpoints), but it did decrease the frequency of dactylitis and painful episodes (secondary outcomes).
Stroke with Transfusions Changing to Hydroxyurea (SWiTCH)	2012	Terminated early due to futility for the primary composite endpoint of recurrent stroke and resolution of iron overload. There was an excess of recurrent strokes in the hydroxyurea arm ($N=7$) compared with continued transfusions ($N=0$).
Stroke (TWiTCH)	2016	For patients placed on blood transfusion therapy for elevated TCD velocities for at least 12 months, switching to maximum tolerated doses of hydroxyurea was noninferior to continued chronic blood transfusion therapy.
A Phase 3 Trial of L-Glutamine in Sickle Cell Disease	2017	L-Glutamine decreased crisis frequency from a median of 3.0 vs 4.0 compared to placebo. There were fewer hospitalizations (median 2.0 vs 3.0) and episodes of acute chest syndrome in the study drug arm compared to placebo.
Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease	2017	Crizanlizumab, a P-selectin inhibitor, decreased crisis rate when compared to placebo (median of 1.63 in study arm vs 2.98 in placebo arm). Time to first crisis was also significantly longer in the crizanlizumab arm than the placebo arm (4.07 months vs 1.38 months).

TCD, transcranial Doppler.

of opioids requires close monitoring because the hypoxia or acidosis resulting from respiratory suppression is particularly dangerous. Meperidine is discouraged because of its short half-life and the accumulation of the toxic metabolite normeperidine, which lowers the seizure threshold. Use of pain assessment instruments and attention to the level of sedation at regular intervals are necessary. Oxygen supplementation is not required unless hypoxemia is present. The use of incentive spirometry has been shown to reduce pulmonary complications in patients presenting with chest or back pain. It has been demonstrated that the number of hospitalizations for painful events can be reduced by prompt intervention in an outpatient setting dedicated to sickle cell disease management. Nonpharmacologic management techniques should be considered, as well as evaluation for depression for the patient with frequent episodes or chronic pain. Blood transfusion is not indicated in the treatment of uncomplicated painful episodes.

Acute chest syndrome

The diagnosis of acute chest syndrome is based on a new radiographic pulmonary infiltrate associated with symptoms such as fever, cough, and chest pain and frequently is not distinguishable from infectious pneumonia. As the nonspecific term implies, various insults or triggers can lead to acute chest syndrome and treatment for infectious pneumonia should be concurrent. Young age, low HbF, high steady-state hemoglobin, and elevated white blood cell count in steady state have been identified as risk factors. In a multicenter prospective study, bacterial (often atypical) or viral infections accounted for approximately 30% of episodes, whereas fat emboli from the bone marrow were responsible for approximately 10% of events, with pulmonary infarction as another common suspected cause. In children, fever is a common presenting symptom, whereas chest pain is more common in adults. Acute chest syndrome often develops in patients who initially present only with an acute painful event. Early recognition of the

condition is of utmost importance because acute chest syndrome has become the leading cause of death for both adults and children with sickle cell disease. Management includes maintaining adequate oxygenation and administration of antibiotics to address the major pulmonary pathogens and community-acquired atypical organisms. Fluid management needs particular attention to prevent pulmonary edema by limiting oral and intravenous hydration to 1.0 to 1.5 times maintenance (after correction of any dehydration). Pain control to avoid excessive chest splinting and the use of incentive spirometry are key adjunctive measures. Bronchodilator therapy is effective if there is associated reactive airway disease, which is particularly common in children. Transfusion of RBCs should be considered if there is hypoxemia or acute symptomatic exacerbation of anemia. Exchange transfusion should be performed for hypoxemia despite oxygen supplementation, widespread (bilateral, multilobar) infiltrates, and rapid clinical deterioration. Patients with acute chest syndrome are at risk for recurrences as well as subsequent chronic lung disease. Preventive measures include hydroxyurea therapy and chronic RBC transfusions.

Central nervous system disease

Without primary prevention, overt stroke may occur in 11% of young sickle cell anemia patients (but is much less common in SC disease and S β^+ -thalassemia), accounting for significant morbidity and mortality. The more frequent use of neuroimaging has identified a substantial incidence of subclinical cerebrovascular disease, with 25% to 40% of children having covert or silent strokes. The majority of overt strokes result from ischemic events involving large arteries with associated vascular endothelial damage, including intimal and medial proliferation. Hemorrhagic events are more common in adults and may result from rupture of collateral vessels (moyamoya) near the site of previous infarction. Suspicion of a neurologic event requires emergent imaging with computed tomography (CT) to assess for hemorrhage followed by MRI. The acute management of overt stroke includes transfusion, usually by an exchange technique, to reduce the HbS percentage to <30% as the pretransfusion target. Chronic transfusion therapy to maintain the HbS <30% decreases the chance of recurrent overt stroke but does not eliminate it. After 3 to 5 years of such transfusions and no recurrent neurologic events, some physicians “liberalize” the transfusion regimen to maintain the HbS <50%. The optimal duration of transfusions is not known, and they often are continued indefinitely. A pediatric randomized controlled trial (the SWiTCH study) of continued chronic transfusions vs hydroxyurea for long-term secondary stroke prevention was

stopped early due to futility, and there was an excess of recurrent strokes in the hydroxyurea arm ($N=7$) compared with continued transfusions ($N=0$).

An abnormally increased TCD blood flow velocity can identify children with HbSS at high risk of primary overt stroke. A randomized controlled trial of prophylactic transfusions vs observation for children with abnormal TCD velocities showed a reduced risk of the first stroke in patients receiving transfusions (the STOP study). The results of a phase 3 primary stroke prevention multicenter randomized controlled trial for children with abnormal TCD velocities (the SWiTCH study) showed that for patients placed on blood transfusion therapy for elevated TCD velocities for at least 12 months, switching to maximum tolerated doses of hydroxyurea was noninferior to continued chronic blood transfusion therapy.

Silent cerebral infarcts (SCIs) are the most common neurologic complications in children with sickle cell anemia. A randomized clinical trial assigned children of ages 5 to 15 years with sickle cell anemia to receive regular blood transfusions or observations. Regular blood-transfusion therapy significantly reduced the incidence of the recurrence of SCIs (6% in treatment arm vs 14% in observation arm). In the STOP-2 trial, patients with normal baseline MRI were assigned to continued transfusions or stopping transfusions. Twenty percent of patients who stopped transfusions developed SCIs.

Notably, the above mentioned randomized clinical trials were all done in patients with SS and S β^0 disease. There is currently no evidence to guide management of SC patients with cerebral vascular accidents and neurologic events, but in general, the approach is extrapolated from the trials in patients with SS and S β^0 disease.

Pregnancy

Pregnancy poses some risk to the mother as well as to the fetus. Spontaneous abortions occur in approximately 5% of pregnancies in sickle cell anemia, and preeclampsia occurs at an increased frequency in sickle cell disease. Low birth weight, preterm labor, and premature delivery are common. All patients should be followed in a high-risk prenatal clinic, ideally at 2-week intervals with close consultation with a hematologist. Patients should receive folic acid 1 mg/d, in addition to the usual prenatal vitamins, and should be counseled regarding the additional risks imposed by poor diet, smoking, alcohol, and substance abuse. Data do not support the routine use of prophylactic transfusions. Simple or exchange transfusions, however, should be instituted for the indications outlined previously, as well as for pregnancy-related complications (eg, preeclampsia). Close follow-up is indicated postpartum when the patient

Table 7-6 Recommended immunizations for patients with sickle cell disease

Specific immunizations for patients with sickle cell disease	Frequency
Influenza vaccination	Annual
Pneumococcal vaccination	PPV-23: At least 2 doses 5 years apart, and then per some guidelines every 5 years after. If only 2 doses are given 5 years apart, a third dose should be given at age 65 years. PCV-13: 1 lifetime dose at age 18 or after
Meningococcal vaccination	Primary dosing depending on age at administration. Regardless of age, the patient should get a quadrivalent conjugate vaccine for at least 2 doses followed by a booster every 5 years. Additional dosing for serogroup B is recommended in some vaccine schedules.
Hepatitis B vaccine	Three doses at 0, 1, and 4 months. Subsequent frequency of antibody screening for continued immunization is not outlined.

is still at high risk for complications. The option of contraception with an intrauterine device, subcutaneous implant, progesterone-only contraceptives, or condoms should be discussed with all women of childbearing age.

RBC transfusion

Patients with sickle cell disease often receive transfusions unnecessarily. RBC transfusions, however, may be effective for certain complications of the disease. Transfusion is indicated as treatment for specific acute events, including moderate to severe acute splenic sequestration, symptomatic aplastic crisis, cerebrovascular accident (occlusive or hemorrhagic), acute ocular vaso-occlusive events, and acute chest syndrome with hypoxemia. Although the first 2 events only require correction of anemia and thus are treated with simple transfusion, stroke, ocular events, and severe acute chest syndrome are best treated with exchange transfusion aimed at decreasing the percentage of HbS to <30% and increasing the Hb level to 9 to 10 g/dL. In addition, transfusions are indicated for the prevention of recurrent strokes as well as for the treatment of high-output cardiac failure. As mentioned, an abnormal TCD velocity can identify children with HbSS and S β ⁰ at high risk of primary overt stroke, which can be prevented by chronic transfusion therapy. Transfusion has also been advocated for patients with severe pulmonary hypertension and chronic nonhealing leg ulcers and to prevent recurrences of priapism, but clinical trial data are lacking. When chronic transfusion is indicated, RBCs may be administered as a partial exchange transfusion, which may offer a long-term advantage of delaying iron accumulation. The goal of chronic transfusion is usually to achieve a nadir total hemoglobin level of 9 to 10 g/dL with the HbS under 30% to 50%. It is important to avoid the hyperviscosity

associated with hemoglobin levels >11 to 12 g/dL in the presence of 30% or more HbS. Patients with HbSC requiring transfusion pose special challenges, with the need to avoid hyperviscosity usually necessitating exchange transfusion (goal HbA >70%) to ensure the hemoglobin concentration does not exceed 11 to 12 g/dL.

Preoperative transfusion in preparation for surgery under general anesthesia may afford protection against perioperative complications and death but is probably not indicated in all cases, particularly minor procedures in children. In a multicenter study, simple transfusion to a total hemoglobin level of 10 g/dL afforded protection equal to partial exchange and was associated with less red cell alloimmunization. Another randomized trial, TAPS Study, included patients with sickle cell anemia undergoing low- or medium-risk surgery. Subjects were randomized to either preoperative transfusion or no transfusion. Thirty-nine percent of 33 patients in the no-preoperative-transfusion group had clinically important complications, compared with 15% in the preoperative-transfusion group ($P=0.023$), leading to early termination due to the number of complications in the nontransfusion arm. Patients undergoing prolonged surgery or with regional compromise of blood supply (eg, during orthopedic surgery), hypothermia, or a history of pulmonary or cardiac disease may do better with preoperative exchange transfusion, although this has not been evaluated in a randomized clinical trial. Transfusions also may be useful for some patients preparing for intravenous ionic contrast studies, dealing with chronic intractable pain, or facing complicated pregnancy. Transfusions are not indicated for the treatment of steady-state anemia, uncomplicated pain events, uncomplicated pregnancy, most leg ulcers, or minor surgery not requiring general anesthesia.

Up to 30% of patients with sickle cell disease who repeatedly undergo transfusion become alloimmunized to RBC antigens (especially E, C, and Kell), and this risk increases with increasing exposure. Alloimmunization predisposes patients to delayed transfusion reactions and possible hyperhemolysis, which can lead to potentially life-threatening anemia and multiorgan failure. Severe painful crises with a decrease in the hemoglobin level within days to weeks of a transfusion should alert the clinician to consider this diagnosis. Identification of a new alloantibody may not be made acutely, and reticulocytopenia can be an associated finding. In this situation, additional transfusions are hazardous and should be avoided if at all possible. Universal RBC phenotyping and matching for the antigens of greatest concern (eg, C, D, E, and Kell) can minimize alloimmunization.

Modifying the disease course

In addition to chronic transfusions, 3 other disease-modifying treatments currently are available: (i) hydroxyurea and (ii) L-glutamine, which are ameliorative; and (iii) hematopoietic stem cell transplantation, which is curative.

Hydroxyurea

On the basis of knowledge that patients with high hemoglobin F levels have less severe disease, many investigators tested a variety of experimental strategies for pharmacologic induction of hemoglobin F production and identified hydroxyurea as efficacious and practical. A multicenter, randomized, placebo-controlled trial then found that daily oral administration of hydroxyurea significantly reduced the frequency of pain episodes, acute chest syndrome, and transfusions in adult HbSS patients (MSH study). No serious short-term adverse effects were observed, although monitoring of blood counts was required to avoid potentially significant cytopenias. Interestingly, the therapeutic response to hydroxyurea sometimes precedes or occurs in the absence of a change in HbF levels, suggesting that a reduction in white blood cell count and other mechanisms may be beneficial. Laboratory studies revealed that hydroxyurea reduced adherence of RBCs to vascular endothelium, improved RBC hydration, and increased the time to polymerization. Follow-up at 17.5 years indicates that patients taking hydroxyurea seem to have reduced mortality without evidence for an increased incidence of malignancy. Classical indications for hydroxyurea include frequent painful episodes, recurrent acute chest syndrome, severe symptomatic anemia, and other severe vaso-occlusive events. Given the safety of hydroxyurea and that HbSS is a morbid condition,

many clinicians use hydroxyurea more liberally even when the classical indications for hydroxyurea therapy are not present. There are now guideline recommendations to strongly consider the use of hydroxyurea in patients with sickle cell anemia who have daily chronic pain that interferes with quality of life. Clinical trials of hydroxyurea in children also show a reduction in the frequency of painful episodes, but no convincing evidence yet indicates that early hydroxyurea therapy prevents or delays the onset of organ damage. Pregnancy should be avoided while taking hydroxyurea. Hydroxyurea should be considered first-line therapy in patients who meet the guideline recommendations for treatment.

L-Glutamine

The role of oxidative stress in the pathophysiology of sickle cell disease is complex. The integrity of the red cell membrane is affected by reactive oxygen species (ROS) generation, with a dose-dependent effect of ROS on membrane rigidity and decreased elasticity. Both red cell and leukocyte adhesion have been shown to increase with superoxide production in sickle cell disease. L-glutamine therapy, which increases the proportion of reduced nicotinamide adenine dinucleotide in sickle red cells and presumably reduces oxidative stress and potentially painful events, was tested in a randomized controlled clinical trial. The randomized study included 230 patients (age 5 to 58 years) with either HgbSS or S β ⁰-thalassamia with a history of 2 or more crises during the previous year. Patients randomized to L-glutamine had significantly fewer sickle cell crises than patients receiving placebo (median 3.0 vs 4.0 crises). Fewer hospitalizations (median 2.0 vs 3.0) and episodes of acute chest syndrome occurred in the study group. The majority of subjects on both arms were on hydroxyurea. L-Glutamine (Endari) was approved by the FDA in 2017. Endari is available in powder form and is mixed with food at doses of 5 to 15 grams based on weight, and given twice daily.

Hematopoietic stem cell transplantation

Allogeneic transplant is curative therapy for people with sickle cell disease. In a recent assessment of outcomes from 3 transplant registries that included 1,000 recipients of HLA-identical sibling transplants performed between 1986 and 2013, the 5-year overall survival for children under 16 years was 95%, with an event-free survival of 93%. For those over the age of 15 years, the overall survival and event-free survival were both 81%. In most centers, few patients meet the usual eligibility criteria, which includes an HLA-matched sibling donor. Questions remain about

who should be referred for transplant and whether asymptomatic people with severe genotypes should be sent for transplant. Results from a Belgian registry found that patients treated with hydroxyurea had improved survival compared to those who underwent transplant. In that study with 15 years of follow-up, mortality rates for hydroxyurea and transplant groups were 0.14 and 0.36 per 100 patient-years, respectively. Longer follow-up might change these numbers and survival is not the only outcome to assess the risk-benefit profile of transplant. Alternative donor sources such as umbilical cord blood, unrelated matched, and haplotype are now being investigated. These alternative donor options and nonmyeloablative conditioning regimens have shown some promise, but remain investigational. As these efforts are undergoing further development, consideration of long-term effects of transplant such as loss of fertility and secondary malignancies, should also be considered.

Selectin inhibition

The potential to interrupt the cell-cell interactions that are thought to be involved in vaso-occlusive events, through selectin inhibition, has garnered much interest. Several clinical trials of selectin inhibition have recently been completed or are under way. Rivipansel is a pan-selectin inhibitor that is being studied in hospitalized patients to treat painful crises. In a phase II study, rivipansel demonstrated a reduction in the mean time to vaso-occlusive event resolution by 28%, and had an 83% reduction in mean cumulative IV opioid analgesic use compared to placebo. Crizanlizumab, a P-selectin inhibitor, was studied in outpatients to prevent vaso-occlusive crises, and decreased crisis rate when compared to placebo (median of 1.63 in study arm vs 2.98 in placebo). Subjects on crizanlizumab also had a prolonged time to first crisis that was significantly longer than those on placebo (4.07 months vs 1.38 months).

CLINICAL CASE (continued)

The case in this section describes a patient with sickle cell anemia who previously experienced pain episodes without major complications related to his disease. He is admitted for a pain crisis, and multiorgan failure ensues. Acute multiorgan failure is a well-described complication of sickle cell disease. High baseline hemoglobin levels may represent a key risk factor. Acute multiorgan failure is often precipitated by a severe acute pain crisis, and is thought to be secondary to widespread intravascular sickling, fat embolization, and subsequent ischemia within affected organs. Aggressive transfusion therapy can be lifesaving and result in complete recovery.

KEY POINTS

- The clinical manifestations of sickle cell disease are primarily due to hemolysis and vaso-occlusion.
- Multiple cellular and genetic factors contribute to the phenotypic heterogeneity of sickle cell disease.
- The hemoglobin F level is a major determinant of clinical manifestations and outcomes.
- Pneumococcal sepsis is now uncommon, but it remains a potential cause of death in infants and young children, so universal newborn screening, compliance with penicillin prophylaxis, and vaccination remain a priority.
- Human parvovirus infection is the cause of aplastic crisis.
- Splenic sequestration should be considered in the differential diagnosis of a sudden marked decrease in the hemoglobin concentration.
- There are differences in frequency of clinical events and survival among the various genotypes of sickle cell disease.
- Sickle cell disease is a leading cause of stroke in young individuals, and a substantial incidence of covert or silent infarctions recently has been appreciated.
- A randomized clinical trial has demonstrated efficacy of red cell transfusion in preventing first stroke in children with abnormal TCD velocity.
- A randomized clinical trial demonstrated that preoperative simple transfusion was as effective as exchange transfusion. The preoperative management of the older patient, particularly with cardiac or pulmonary dysfunction, has not been defined.
- A randomized, placebo-controlled clinical trial has established the efficacy of hydroxyurea in reducing the frequency of painful episodes and acute chest syndrome. A follow-up study suggests a reduction in mortality for patients taking hydroxyurea.
- The causes of acute chest syndrome include infection, fat embolism, and pulmonary infarction.

Other hemoglobinopathies

Hemoglobin E

HbE is a β -chain variant with highest frequency in Southeast Asia. The highest prevalence occurs in Myanmar and Thailand, where the gene frequency may approach 70% in certain regions. The gene frequency is also high in Laos, Cambodia, and Vietnam. It is also found in Sri Lanka, northeastern India, Nepal, Bangladesh, Malaysia, Indonesia, and the Philippines. It has become more common in the United States during the past 20 to 30 years as a result of immigration. The structural change is a substitution of glutamic acid by lysine at the 26th position of

the β -globin chain (Figure 7-7). The mutation is also thalassemic because the single-base GAG/AAG substitution creates a cryptic splicing site, which results in abnormal mRNA processing and reduction of mRNA that can be translated. HbE is also slightly unstable in the face of oxidant stress and is sometimes referred to as a “thalassemic hemoglobinopathy.”

Individuals with hemoglobin E trait are asymptomatic with or without mild anemia (hemoglobin >12 g/dL), and mild microcytosis. Peripheral smear may be normal or may show hypochromia, microcytosis, target cells, irregularly contracted cells, and basophilic stippling. HbE usually makes up 30% or less of total hemoglobin. The HbE concentration is lower with the coinheritance of α -thalassemia. Homozygotes (HbE disease) are usually asymptomatic with no overt hemolysis or splenomegaly. Individuals may have mild anemia, microcytosis (MCV approximately 65 to 69 fL in adults and 55 to 65 fL in children), and reduced MCH. Peripheral smear shows hypochromia, microcytosis, and a variable number of target cells and irregularly contracted cells. HbE plus HbA₂ makes up 85% to 99% of the total hemoglobin. The compound heterozygous state, HbE β -thalassemia, results in a very variable phenotype ranging from thalassemia trait, NTDT, to TDT, depending on the β -mutation. It is now one of the more common forms of thalassemia in the United States. It is characterized by microcytic anemia, with mildly increased reticulocytosis. The peripheral smear includes anisocytosis, poikilocytosis, hypochromia, microcytosis, target cells, nucleated red blood cells, and irregularly contracted cells. HbE β^0 -thalassemia is associated with a mostly HbE electrophoretic pattern, with increased amounts of HbF and HbA₂. The electrophoretic pattern in HbE β^+ -thalassemia is similar except for the presence of approximately 15% HbA. HbE comigrates with HbC and HbA₂ on cellulose acetate electrophoresis and isoelectric focusing. HPLC separates HbC, HbA₂ and HbE.

Patients with HbE disease are usually asymptomatic and do not require specific therapy. However, patients who coinherited HbE and β -thalassemia, especially those with HbE- β^0 , may have significant anemia. Some need intermittent or chronic RBC transfusions, and some may benefit from splenectomy.

Hemoglobin C

HbC is the third most common mutant hemoglobin, after HbS and HbE. The HbC mutation arose in West Africa. The prevalence in African Americans is 2% to 3%. The hemoglobin mutant results from the substitution of lysine for glutamic acid at the sixth amino acid of β -globin, the consequence of a single nucleotide substitution (GAG/

AAG) in the sixth codon (Figure 7-7). The resultant positive-to-negative charge difference on the surface of the HbC tetramer results in a molecule with decreased solubility in both the oxy and deoxy forms, which may undergo intraerythrocytic aggregation and crystal formation. HbC stimulates the K:Cl cotransport system, promoting water loss and resulting in dehydration and poorly deformable RBCs that have a predilection for entrapment within the spleen. Consequently, patients with HbCC and patients with HbC β -thalassemia have mild chronic hemolytic anemia and splenomegaly. Patients may develop cholelithiasis, and the anemia may be more exaggerated in association with infections. Heterozygous individuals (HbC trait) are clinically normal; however, identifying the diagnosis is important for genetic counseling. The coinheritance of HbS and HbC results in a form of sickle cell disease, HbSC (see the section “Sickle cell disease” in this chapter).

Laboratory studies in HbCC show a mild hemolytic anemia, microcytosis, and slightly elevated reticulocyte counts. The MCHC is elevated because of the effect of HbC on cellular hydration. The peripheral blood smear shows prominent target cells, microcytosis, and irregularly contracted red cells. RBCs containing hemoglobin crystals also may be seen on the blood smear, particularly in patients who have had splenectomy. Individuals with HbC trait have normal hemoglobin levels, and microcytosis is common. The peripheral smear may be normal or may show microcytosis and target cells. Confirmation of the diagnosis requires identification of HbC; which comigrates with HbA₂, HbE, and HbO^{Arab} on cellulose acetate electrophoresis and isoelectric focusing. Thus, HbC must be distinguished by citrate gel electrophoresis or HPLC.

Specific treatment for patients with HbCC is not generally necessary.

Hemoglobin D

HbD is usually diagnosed incidentally. HbD^{Punjab} (also called HbD^{Los Angeles}) results from the substitution of glutamine for glutamic acid at the 121st position of the β -chain (Figure 7-7). This mutant has a prevalence of approximately 3% in the Northwest Punjab region of India, but is also encountered in other parts of the world. Patients who are homozygous (HbDD) may have a mild hemolytic anemia. Individuals who are heterozygous (HbAD) are clinically normal, with normal blood counts and a peripheral smear with the occasional target cells. The major clinical relevance of HbD is with compound heterozygous inheritance with HbS, resulting in a form of sickle cell disease, perhaps as a result of the low affinity of HbD promoting hemoglobin deoxygenation. The diagnosis of HbAD

(D trait) or HbDD is made by hemoglobin electrophoresis. HbS and HbD have similar electrophoretic mobility on alkaline cellulose acetate electrophoresis and isoelectric focusing. They can be differentiated by acid citrate agar electrophoresis, HPLC, or solubility studies. This distinction is important for genetic and prognostic counseling.

KEY POINTS

- Hemoglobins C, D, and E are common hemoglobin variants that can have significant consequences when coinherited with hemoglobin S.
- Homozygosity for hemoglobin E (EE) is a mild condition, but compound heterozygosity for HbE and β-thalassemia can be a clinically significant thalassemia syndrome.

Unstable hemoglobin

Unstable hemoglobin variants are inherited in an autosomal dominant pattern, and affected individuals are usually heterozygotes. Unstable hemoglobins constitute one of the largest groups of hemoglobin variants, although individually, each is rare. In both Hb Köln (β_{98} Val/Met substitution) and in Hb Zurich (β_{63} His/Arg), the amino acid substitution destabilizes the heme pocket. Other mechanisms that destabilize hemoglobin include (i) alteration of the $\alpha_1\beta_1$ interface region (eg, Hb Tacoma, β_{30} Arg/Ser); (ii) distortion of the helical configuration of structurally important regions (eg, Hb Bibba, α_{136} Leu/Pro); and (iii) introduction of the interior polar amino acid (eg, Hb Bristol, β_{67} Val/Asp). Unstable γ-chain variants (eg, Hb Poole, γ_{130} Trp/Gly) can cause transient hemolytic anemia in the neonate that spontaneously resolves.

These abnormal hemoglobins precipitate spontaneously or with oxidative stress. Precipitated hemoglobin inclusions (Heinz bodies seen using a supravital stain) impair erythrocyte deformability, resulting in premature erythrocyte destruction by macrophages of the liver and spleen. The severity of the hemolysis varies with the nature of the mutation but may be accelerated by fever or ingestion of oxidant drugs.

An unstable hemoglobinopathy should be suspected in a patient with hereditary nonspherocytic hemolytic anemia. The hemoglobin level may be normal or decreased. Hypochromia of the RBCs (resulting from loss of hemoglobin due to denaturation and subsequent pitting), “bite cells,” and basophilic stippling may occur. The evaluation includes hemoglobin electrophoresis (which is often normal), crystal violet Heinz-body staining, and the isopropanol stability test. The isopropanol test may be falsely posi-

tive in the neonate due to high fetal hemoglobin levels, so the heat stability test should be used during the first months of life. Management includes avoidance of oxidant agents, and some recommend supplementation with folic acid. Splenectomy may be useful for patients with severe hemolysis and splenomegaly. The risk of thrombosis is high after splenectomy in individuals with a severely unstable hemoglobin, and thus, patients should be educated and closely evaluated in this regard.

Some unstable hemoglobins may also have altered oxygen affinity, which could exacerbate (decreased oxygen affinity) or ameliorate (increased oxygen affinity) the degree of anemia.

Methemoglobinemia

Methemoglobinemia is characterized by a decrease in hemoglobin's oxygen carrying capacity due to oxidation of the iron moieties in hemoglobin from ferrous (Fe^{2+}) to ferric (Fe^{3+}), which is unable to bind and transport oxygen. High methemoglobin levels cause a functional anemia. Methemoglobinemia can result from either congenital or acquired processes. Congenital forms are due to (i) autosomal dominant mutations in α or β globin chains, producing variants collectively called hemoglobin M, or (ii) autosomal recessive defects in the enzyme cytochrome b_5 reductase (CYB5R). Acquired methemoglobinemia is much more common, and due to exposure to substances that cause oxidation of hemoglobin, including direct oxidizing agents (eg, benzocaine), indirect oxidants (eg, nitrates) or metabolic activation (eg, dapsone). Methemoglobinemia should be considered in the setting of dyspnea, cyanosis, and hypoxemia that is refractory to supplemental oxygen. The clinical presentation is variable depending on the percentage of methemoglobin, rate of methemoglobin accumulation, rate of clearance, and magnitude of exposure. The clinical spectrum includes cyanosis, pallor, weakness, fatigue, headache, metabolic acidosis, dysrhythmias, seizures, central nervous system depression, coma, and death. Generally, the higher the methemoglobin level, the more severe the clinical symptoms. Clinical evaluation for refractory hypoxemia, chocolate-colored blood, arterial or venous blood gas with cooximetry, and determination of methemoglobin percentage are key clues. Treatment for acquired methemoglobinemia generally includes removal of the inciting agent, use of methylene blue, and high flow oxygen to enhance natural degradation of methemoglobin.

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Hemolytic anemias excluding hemoglobinopathies

RONALD S. GO AND KEVIN H. M. KUO

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Introduction

Hemolysis is the accelerated destruction of red blood cells (RBCs), leading to decreased RBC survival. The bone marrow's response to hemolysis is increased erythropoiesis, reflected by reticulocytosis. If the rate of hemolysis is modest and the bone marrow is able to completely compensate for the decreased RBC life span, the hemoglobin concentration may be normal; this is called fully compensated hemolysis. If the bone marrow is unable to completely compensate for hemolysis, then anemia occurs. This is called incompletely compensated hemolysis. Thus, a hemoglobin value within the normal range does not necessarily denote the absence of hemolysis.

Clinically, hemolytic anemia produces variable degrees of fatigue, pallor, and jaundice. Splenomegaly occurs in some conditions. The complete blood count may show anemia and reticulocytosis that depend on the acuity and severity of hemolysis, and the degree and ability of the bone marrow to compensate for the hemolysis. Secondary chemical changes include indirect hyperbilirubinemia, increased urobilinogen excretion, and elevated lactate dehydrogenase (LDH). Decreased serum haptoglobin levels are common and increased plasma-free hemoglobin may also be detected. Because free hemoglobin scavenges nitric oxide (NO) and release of RBC arginase impairs NO bioavailability, erectile dysfunction, esophageal spasm, renal insufficiency or vascular sequelae such as nonhealing skin ulcers and pulmonary hypertension can occur in chronic hemolytic anemia (Figure 8-1). Chronic intravascular hemolysis produces hemosiderinuria, and chronic extravascular hemolysis increases the risk of pigmented (bilirubinate) gallstones. Some hemolytic conditions are also associated with increased risk of thrombosis, due to abnormal red blood cell (RBC) properties, increased plasma concentrations of microparticles, release of cell-free hemoglobin, increased reactive oxygen species, and endothelial dysfunction (Figure 8-1). Iron overload is observed in many cases of congenital hemolytic anemias even in the absence of chronic transfusion, the mechanism of which has not been fully elucidated.

The hemolytic anemias can be classified and approached in different yet complementary ways (Table 8-1). They can be inherited (eg, sickle cell disease or hereditary spherocytosis) or acquired (eg, autoimmune or microangiopathic). They can sometimes be distinguished via a thorough history including

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Off-label drug use: Azathioprine, chlorambucil, cyclophosphamide, cyclosporine, danazol, intravenous immunoglobulin, mycophenolate mofetil, and rituximab in the treatment of autoimmune hemolytic anemia.

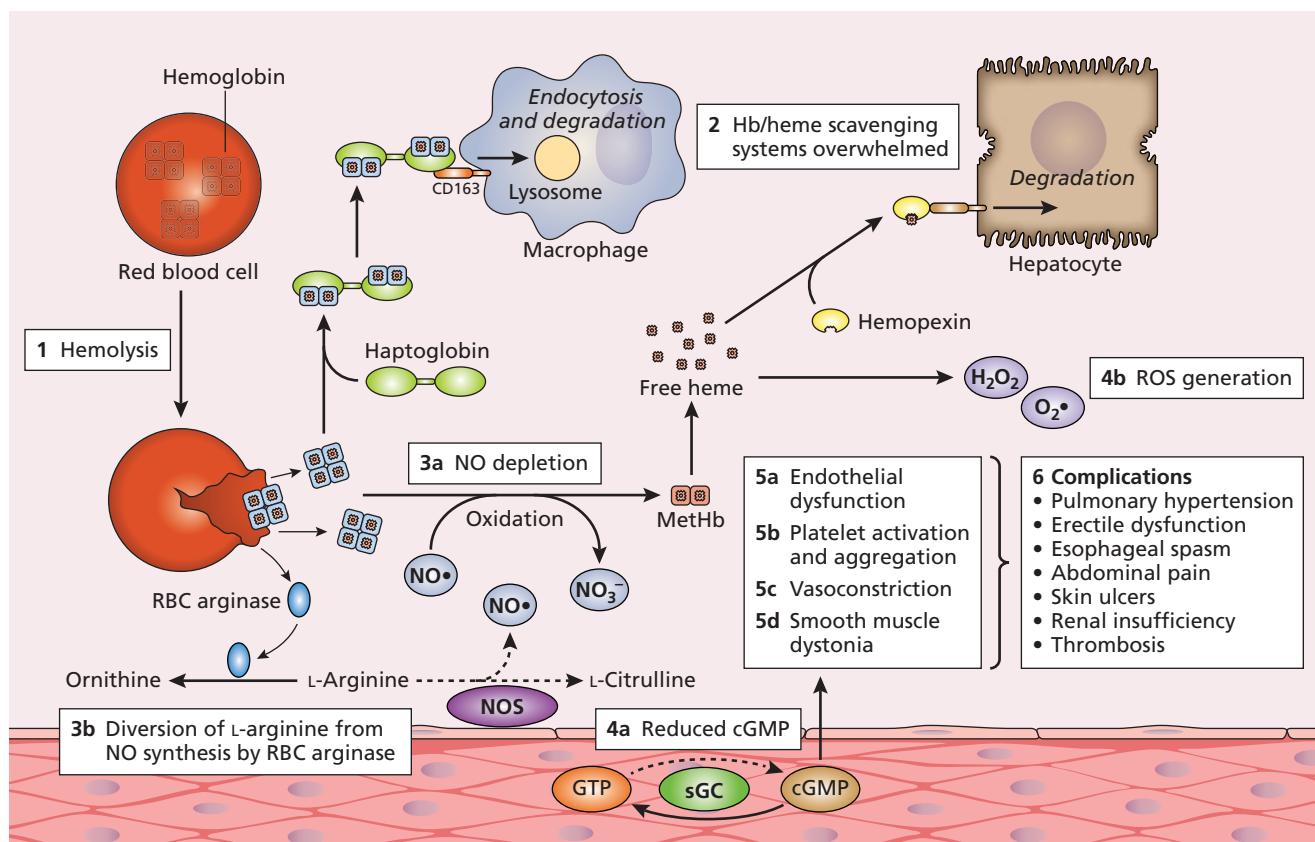


Figure 8-1 The pathophysiology of complications from chronic intravascular hemolysis. Hb, hemoglobin; MetHb, methemoglobin; NOS, nitric oxide synthase; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase. Adapted from Schechter AN, *Blood*. 2008;112(10):3927–3938; Gladwin MT, Ofori-Acquah SF, *Blood*. 2014;123(24):3689–3690; and Schaer DJ et al, *Front Physiol*. 2014;5:415.

the tempo of chronic hemolysis (episodic versus chronic), time of onset of hemolytic complications, prior blood counts and transfusions, antecedent factors leading to the hemolytic episode, and the kinetics in the fall in hemoglobin.

Alternatively, they can be characterized by the anatomic site of RBC destruction: extravascular or intravascular. Extravascular hemolysis, in which erythrocyte destruction occurs by macrophages in the liver and spleen, is more common. Intravascular hemolysis refers to RBC destruction occurring primarily within blood vessels. The distinction between intravascular and extravascular hemolysis is not absolute because both occur simultaneously (at least to some degree) in the same patient, and the manifestations of both can overlap. The site of RBC destruction in different conditions can be conceptualized to occur in a spectrum between pure intravascular and pure extravascular hemolysis. Some hemolytic anemias are predominantly intravascular (eg, paroxysmal nocturnal hemoglobinuria

[PNH]), and some are predominantly extravascular (eg, hereditary spherocytosis [HS]). Others have substantial components of both, such as sickle cell disease. While high LDH and low haptoglobin levels are commonly seen in both intravascular and extravascular hemolysis, these values can be normal in extravascular hemolysis. The presence of hemoglobinuria or hemosiderinuria suggests intravascular hemolysis.

The hemolytic anemias can also be classified according to whether the cause of hemolysis is intrinsic or extrinsic to the RBC. Intrinsic causes of hemolysis include abnormalities in hemoglobin structure or function, the RBC membrane, or RBC metabolism (cytosolic enzymes). Extrinsic causes may be due to an RBC-directed antibody, a disordered vasculature, or the presence of infecting organisms or toxins. In general, intrinsic causes of hemolysis are inherited and extrinsic causes are acquired, but there are notable exceptions. For example, PNH is an acquired intrinsic RBC disorder, and congenital thrombotic

Table 8-1 Methods of classification of hemolytic anemias

Classification	Example
Inheritance	
Inherited	Sickle cell anemia
Acquired	Autoimmune hemolytic anemia
Site of RBC destruction	
Intravascular	Paroxysmal nocturnal hemoglobinuria
Extravascular	Hereditary spherocytosis
Origin of RBC damage	
Intrinsic	Pyruvate kinase deficiency
Extrinsic	Thrombotic thrombocytopenic purpura

thrombocytopenia purpura (TTP) is an inherited cause of extrinsic hemolysis. Peripheral blood film may provide morphologic clues to the diagnosis.

In this chapter, hemolytic anemias are divided into those that are due to intrinsic or extrinsic abnormalities of the RBC. Hemoglobinopathies are covered in Chapter 7.

Hemolysis due to intrinsic abnormalities of the RBC

Intrinsic causes of hemolysis include abnormalities of hemoglobin structure or function, the RBC membrane, or RBC metabolism (cytosolic enzymes). Most intrinsic forms of hemolysis are inherited conditions.

Abnormalities of the RBC membrane

CLINICAL CASE

A 36-year-old woman is referred for evaluation of moderate anemia. She has been told she was anemic as long as she can remember, and she has intermittently been prescribed iron. She occasionally has mild fatigue but is otherwise asymptomatic. Her past history is significant for intermittent jaundice and a cholecystectomy for gallstones at age 22 years. She takes no medications. Her paternal cousin and paternal aunt also have anemia and jaundice. Her examination is significant for mild splenomegaly. Prior laboratory data reveal hemoglobin values between 80 and 110 g/L. Today's hemoglobin is 90 g/L, mean corpuscular volume (MCV) 98 fL, mean corpuscular hemoglobin concentration (MCHC) 380 g/L. The absolute reticulocyte count is $252 \times 10^9/L$. Review of the peripheral blood smear reveals numerous spherocytes.

HS and hereditary elliptocytosis/hereditary pyropoikilocytosis (HE/HPP) are a heterogeneous group of hemolytic disorders with a wide spectrum of clinical manifestations. HS and HE/HPP are characterized by abnormal shape and flexibility of RBCs because of a deficiency or dysfunction of 1 or more of the membrane proteins, while overhydrated (OHS), dehydrated hereditary stomatocytosis (DHS), and cryohydrocytosis affect cation permeability of the RBC membrane. Multiple genetic abnormalities, including deletions, point mutations, and defective mRNA processing, have been identified as underlying causes.

RBC membrane protein composition and assembly

The RBC membrane consists of a phospholipid-cholesterol lipid bilayer intercalated by integral membrane proteins, including the band 3 macrocomplex (band 3, Rh protein, Rh-associated glycoprotein [RhAG], and CD47) and the glycophorins (Figure 8-2). This relatively fluid layer is stabilized by attachment to a membrane skeleton. Spectrin is the major protein of the skeleton, accounting for approximately 75% of its mass. The skeleton is organized into a hexagonal lattice. The hexagon arms are formed by fiberlike spectrin tetramers, whereas the hexagon corners are composed of small oligomers of actin that, with the aid of other proteins (4.1 and adducin), connect the spectrin tetramers into a 2-dimensional lattice. The membrane cytoskeleton and its fixation to the lipid-protein bilayer are the major determinants of the shape, strength, flexibility, and survival of RBCs. When any of these constituents is altered, RBC survival may be shortened.

A useful model to understand the basis for RBC membrane disorders divides membrane protein-protein and protein-lipid associations into 2 categories. Vertical interactions are perpendicular to the plane of the membrane and involve a spectrin-ankyrin-band 3 association facilitated by protein 4.2 and attachment of spectrin-actin-protein 4.1 junctional complexes to glycophorin C (Figure 8-2). Horizontal interactions, which are parallel to and underlying the plane of the membrane, involve the assembly of α - and β -spectrin chains into heterodimers, which self-associate to form tetramers (Figure 8-2). Because the distal ends of spectrin bind to actin, with the aid of protein 4.1 and other minor proteins, a contractile function of the cytoskeleton may be important for normal RBC survival. Conceptually, HS is caused by defects in vertical protein-protein interactions in the RBC membrane, whereas HE/HPP is caused by defects in horizontal interactions.

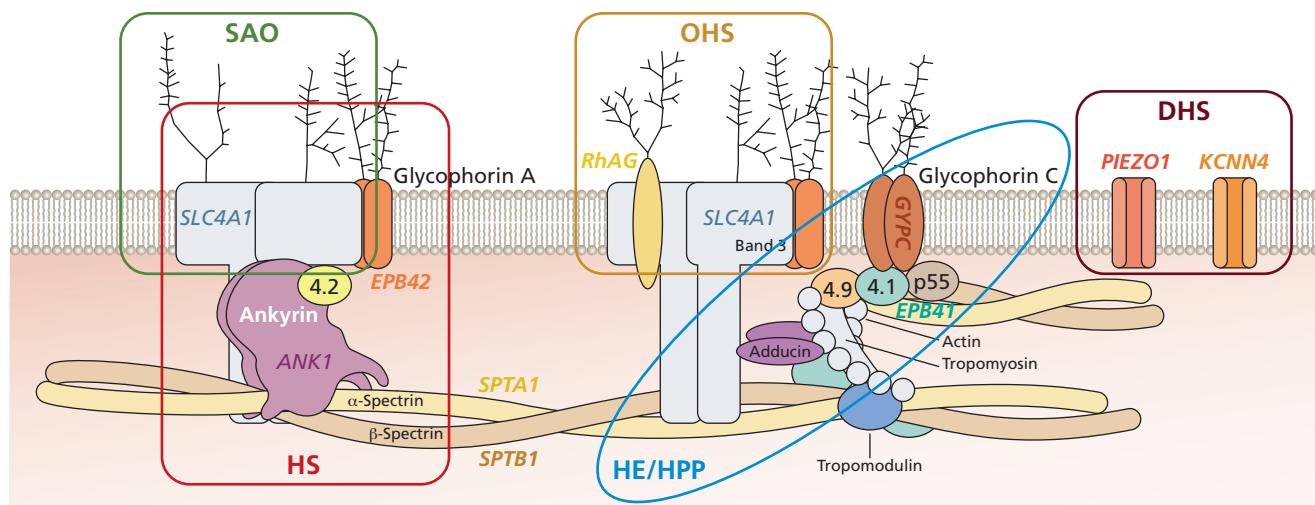


Figure 8-2 Red blood cell membrane cytoskeletal structure and the associated membranopathies, with the corresponding genes responsible encircled. Mutations from different cytoskeletal genes can give rise to the same phenotype, while certain proteins, such as band 3, can give rise to different phenotypes depending on the location and nature of the mutation. Note that HS and HE/HPP are caused by defects in protein-protein interactions in the vertical and horizontal axes, respectively. HS, hereditary spherocytosis; HE, hereditary elliptocytosis; SAO, Southeast Asian ovalocytosis; OHS, overhydrated hereditary stomatocytosis; DHS, dehydrated hereditary stomatocytosis. Modified from Liem R, Gallagher PG. *Drug Discov Today Dis Mech*. 2(4):539.

Hereditary spherocytosis

HS is common in individuals of northern European descent with an occurrence of approximately 1 in 2,000 to 5,000 births. Penetrance is variable, and the prevalence of a clinically recognized disorder is much lower. In 75% of cases, the inheritance pattern is autosomal dominant with sporadic cases representing the remaining 25%, half of which represent an autosomal recessive inheritance pattern and the other half de novo mutations. The HS syndromes generally are due to private mutations unique to each kindred. HS is characterized by spherocytic, osmotically fragile RBCs and is both clinically and genetically heterogeneous (Figure 8-3a).

Pathophysiology

The pathophysiology of HS generally involves aberrant interactions between the skeleton and the overlying lipid bilayer (vertical interactions). A common epiphénoménon in HS RBCs is a varying degree of spectrin loss, which is usually due to a defect in one of the membrane proteins involved in the attachment of spectrin to the membrane rather than a primary defect in the spectrin molecule itself. Spectrin as the major protein of the skeleton forms a nearly monomolecular submembrane layer that covers most of the inner-membrane surface; therefore, the density of this skeletal layer in HS erythrocytes is reduced. Consequently, the lipid bilayer is destabilized, leading to

loss of membrane lipid and thus surface area through microvesiculation. The result of these changes is a progressively spheroidal RBC. The inherent reduced deformability of spherocytes makes it difficult for them to traverse the unique constraining apertures that characterize splenic vascular walls. The spleen “conditions” RBCs, enhancing membrane loss. Retained and further damaged by the hypoxic and acidic environment in the spleen, they ultimately are destroyed prematurely.

The molecular basis of HS is heterogeneous (Table 8-2). A deficiency or defect of the ankyrin molecule represents the most common cause of dominant HS. In 30% to 45% of cases, the defect includes both ankyrin and spectrin deficiency; in 30% spectrin only, and in 20% band 3 mutations. Various mutations of the ankyrin gene have been identified. Multiple band 3 mutations have been described. Although less frequent, mutations of the β -spectrin gene have been found in autosomal dominant HS, whereas α -spectrin gene abnormalities have been identified only in recessively inherited HS. Mutations in the protein 4.2 gene have been found primarily in Japanese patients with autosomal recessive HS.

Clinical manifestations

The clinical expression ranges from an asymptomatic and often undiagnosed condition with nearly normal hemoglobin levels (compensated hemolysis) to severe

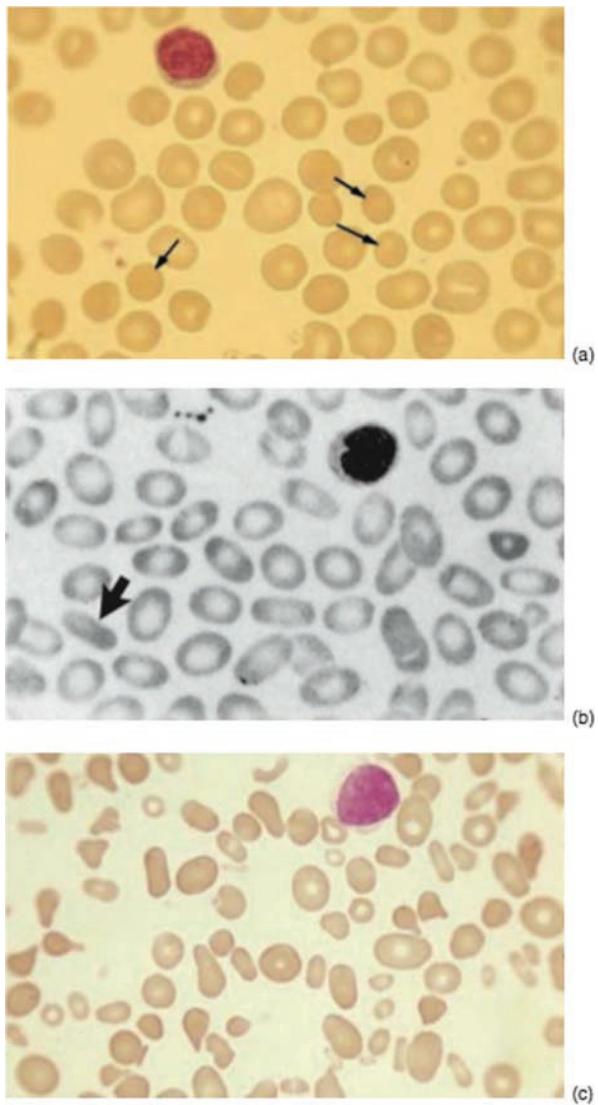


Figure 8-3 Peripheral blood findings in inherited disorders of the red cell membrane. (a) Numerous spherocytes (arrows); (b) numerous elliptocytes and a rod-shaped cell (arrow); (c) marked poikilocytosis.

hemolysis and anemia. Patients with mild HS have a relatively uneventful course, although some may develop pigmented gallstones in childhood or adult life. Mildly anemic patients may be diagnosed later in life as adults during evaluation for unrelated conditions. Patients with moderately severe disease may present with several additional complications. Aplastic crisis, which may be the initial presentation for some patients, may require urgent attention. The cause of aplastic crisis is human parvovirus infection, which produces selective suppression of erythropoiesis, resulting in reticulocytopenia and inability to

compensate for ongoing RBC destruction. In contrast, the “hyperhemolytic crisis” is characterized by accelerated hemolysis, leading to increased jaundice and splenic enlargement, which is a common problem in children. Other complications include the rare megaloblastic crisis secondary to acquired folic acid deficiency, usually associated with high-demand situations such as pregnancy. Leg ulcerations have been rarely reported. Patients with severe hemolysis and resulting expansion of the erythroid compartment in the bone marrow can develop maxillary hyperplasia interfering with dentition or extra-medullary hematopoietic masses that may mimic malignancy. Iron overload is a chronic complication of HS, even in non-transfusion-dependent patients. Patients may manifest a variety of issues attributable to splenomegaly, including early satiety, left upper-quadrant fullness, and hypersplenism. HS may be diagnosed in the neonatal period based on a positive family history or marked jaundice. The diagnosis also should be considered in patients of all ages with intermittent jaundice, mild “refractory” anemia, or splenomegaly. Rare associated syndromes suggest that mutant RBC membrane proteins may reside in other tissues. For example, distal renal tubular acidosis may occur in HS patients harboring mutant band 3 (the anion channel protein).

Laboratory evaluation

In addition to the usual laboratory abnormalities indicating hemolysis, the principal diagnostic feature is the identification of spherocytes on the peripheral blood smear (Figure 8-3a). The extent of spherocytosis is variable, and in mild cases, it may be missed even by the experienced clinician. Additional morphologic abnormalities, including cells with membrane extrusions and elliptocytes, may be observed. The RBC indices may provide a clue, with an increase in the MCHC (due to cellular dehydration) even in the context of minimal anemia. Review of the complete blood count, reticulocyte count, and peripheral smear from family members may prove helpful. The differential diagnosis for spherocytes includes autoimmune and drug-induced hemolytic anemia, so exclusion of these causes and a direct antiglobulin test (DAT) should be performed as part of the evaluation when the family history is negative. Likewise, HS should be considered in the differential diagnosis of DAT-negative hemolytic anemia.

Several specialized diagnostic tests are available to diagnose and distinguish different membranopathies. Eosin-5-maleimide (EMA) binding assay relies on EMA binding to band 3 on RBCs, and a reduction in binding, measured

Table 8-2 Defects of red blood cell membrane proteins in hereditary spherocytosis, elliptocytosis, and pyropoikilocytosis

Class of defect	Hereditary spherocytosis	Hereditary elliptocytosis and pyropoikilocytosis
Protein deficiency (gene)	Spectrin (<i>SPTA1, SPTB1</i>)	Spectrin [†] (<i>SPTA1, SPTB1</i>)
	Ankyrin* (<i>ANK1</i>)	Protein 4.1 (<i>EPB41</i>)
	Band 3 (<i>SLC4A1</i>)	Glycophorin C (<i>GYPC</i>)
	Protein 4.2 (<i>EPB42</i>)	
Protein dysfunction	β-spectrin abnormality affects β-spectrin–protein 4.1 interaction*	Defective spectrin dimer self-association due to spectrin mutations
		Protein 4.1 abnormality affects β-spectrin–protein 4.1 interaction

*Red cells of these patients are also partially deficient in spectrin.

[†]Seen in patients with hereditary pyropoikilocytosis in cases in which it coexists with a spectrin mutation that affects spectrin self-association.

by fluorescence intensity, corresponds to a quantitative reduction in erythrocyte band 3 or the band 3 complex. The cryohemolysis test utilizes an increased susceptibility of HS red cells to rapid cooling from 37°C to 0°C in hypertonic conditions. Osmotic gradient ektacytometry measures deformability of whole RBCs as a function of osmolality using a laser-diffraction viscometer, and it can help differentiate HS from HE/HPP and stomatocytosis. The osmotic fragility test (OFT) using increasingly hypotonic saline solutions supports the diagnosis with the finding of increased RBC lysis compared with normal RBCs. Sensitivity of the test is enhanced by 24-hour incubation at 37°C, but mild cases still can be missed by the test. EMA binding assay and cryohemolysis test are the recommended screening test for cases that are equivocal. EMA binding assay has better specificity and sensitivity than OFT and is comparable to ektacytometry.

Treatment

As with other hemolytic anemias, folic acid supplementation should be considered for patients with severe anemia, even though overt folic acid deficiency rarely is encountered in the industrial nations due to supplementation in grain products. Patients need to be aware of the signs and symptoms of aplastic and hyperhemolytic crises to seek prompt medical attention. The definitive treatment of HS is splenectomy, which ameliorates the hemolytic anemia in almost all patients, although the underlying intrinsic defect of the circulating RBCs is not altered. Dehydrated hereditary stomatocytosis (DHS) must be ruled out prior to splenectomy as it is contraindicated in DHS (see section on hereditary stomatocytosis). In rare patients with HS and severe hemolysis, splenectomy markedly diminishes the hemolytic rate but may not fully correct the

anemia. Clinical trial data are not available to provide guidelines in making the decision to recommend splenectomy. Thus, the indications for splenectomy are somewhat controversial, but the prevailing view advocates splenectomy for patients with symptomatic hemolytic anemia or its complications. Additional considerations for splenectomy in the pediatric population include failure to thrive, recurrent hyperhemolytic episodes, or complications of chronic anemia, including a hypermetabolic state. The laparoscopic technique often is preferred to open splenectomy. Accessory spleens are common, so a thorough search should be performed at the time of splenectomy. The patient should receive pneumococcal, *H. influenzae* type b, and meningococcal vaccines before the procedure, and pediatric patients usually receive prophylactic penicillin for at least several years thereafter to reduce the risk of bacterial sepsis. Thromboembolic events may occur following splenectomy, although data are limited. Because of the increased frequency of post-splenectomy infections in young children, splenectomy should not be performed before the age of 5 years except in patients with particularly severe disease. Partial splenectomy has been advocated to resolve the anemia of HS yet maintain some residual splenic phagocytic function. Long-term results of partial splenectomy (4 to 6 years) in small observational studies are promising, but the spleen may increase in size and the hemoglobin concentration may fall after splenectomy. Markers of splenic function indicate variable degrees of residual activity, but postoperative penicillin is recommended. Iron overload can be treated with iron chelation or phlebotomy but must be weighed against the side effect of iron chelation (see Table 5-4), the individual's ability to tolerate phlebotomy, and potential stimulation or exacerbation of extramedullary hematopoiesis from phlebotomy.

CLINICAL CASE

The patient presented in this section and her father are found to have HS. Her father remains asymptomatic. It is not uncommon for the diagnosis to be made in adulthood, as patients with mild or moderate disease are often well compensated. An elevated reticulocyte count, elevated MCHC, intermittent jaundice, history of gallstones, a negative DAT, and spherocytes on peripheral smear all support the diagnosis. Genetic testing can be performed to confirm the presence of a HS-associated mutation. Family members should be evaluated for anemia.

KEY POINTS

- HS is the most common inherited hemolytic anemia of individuals from Northern Europe.
- Abnormalities in ankyrin, spectrin, band 3, and protein 4.2 ("vertical interactions") that result in a reduction in the quantity of spectrin account for the red cell membrane loss characteristic of HS.
- HS should be suspected in cases of direct antiglobulin test-negative hemolytic anemia when spherocytes are identified on the peripheral blood smear. A positive family history is supportive of the diagnosis.
- Clinical manifestations of HS vary from a lack of symptoms to severe hemolysis.
- Splenectomy decreases hemolysis and reduces gallstone formation, but it should be reserved for symptomatic patients.

Hereditary elliptocytosis and hereditary pyropoikilocytosis

The clinical presentation, inheritance, and alteration in RBC shape and physical properties and the underlying molecular defects are considerably more heterogeneous in HE/HPP than in HS. Three distinct subtypes are distinguished: (1) common HE, characterized by biconcave elliptocytes, and in more severe forms as HPP with rod-shaped cells, poikilocytes, and fragments (Figure 8-3b); (2) spherocytic HE, a phenotypic hybrid between HE and HS; and (3) Southeast Asian ovalocytosis with unique spoon-shaped erythrocyte morphology. In most cases, the inheritance of HE is autosomal dominant while HPP is recessively inherited (Figure 8-3c). Some HE/HPP syndromes are due to specific mutations in individuals from similar locales (eg, Melanesian elliptocytosis), suggesting a founder effect. Clinical manifestations range from

asymptomatic carrier state to severe transfusion-dependent hemolytic anemia with poikilocytosis and erythrocyte fragmentation.

Pathophysiology

The underlying defects involve horizontal interactions between proteins of the membrane skeleton, especially spectrin-spectrin and spectrin–protein 4.1 interactions. These defects weaken the skeleton. Under the influence of shear stress in the microcirculation, the cells progressively lose the ability to regain the normal disc shape and are stabilized in the elliptocytic or poikilocytic shape. In severely affected patients, the weakening of the skeleton grossly diminishes membrane stability, leading to RBC fragmentation.

Different underlying molecular defects have been identified in common HE, consistent with the heterogeneous nature of the disorder (Table 8-2). In the majority of cases, patients have mutant α - or β -spectrin, resulting in defective self-association and an increased percentage of spectrin heterodimer in the membrane. A partial or complete absence or dysfunction of protein 4.1 occurs in some patients with missense and deletion mutations. Patients with HPP appear to be compound heterozygotes. Coinheritance of a mutation leading to spectrin deficiency and a mutation of spectrin resulting in a qualitatively defective molecule has been identified in some patients with the condition. Southeast Asian ovalocytosis is prevalent among certain ethnic groups in Malaysia, the Philippines, Papua New Guinea, and probably other Pacific countries as well. It is an asymptomatic condition characterized by rigid RBCs of a unique spoon-shaped morphology. Affected individuals are heterozygous for a mutation of band 3.

Clinical manifestations, laboratory evaluation, and treatment

HE/HPP must be differentiated from a variety of other conditions in which elliptocytes and poikilocytes commonly are found on the peripheral blood smear, including iron deficiency, thalassemia, megaloblastic anemia, myelofibrosis, and myelodysplasia. As opposed to HE/HPP, however, the percentage of elliptocytes in these other conditions usually does not exceed 60%. The presence of elliptocytes and evidence of dominant inheritance of elliptocytosis in other family members differentiate HE/HPP from the previous conditions. In cases where the proband has a severe clinical phenotype but the parents have mild diseases, it is usually caused by a hypomorphic allele (eg, spectrin α^{LELY} allele) coinherited in-trans to a structural spectrin defect allele. Whereas most patients with com-

mon HE/HPP are asymptomatic, occasional patients who are homozygotes or compound heterozygotes for 1 or 2 molecular defects have more severe hemolytic disease. African American neonates with common HE may have severe hemolysis, with striking RBC abnormalities similar to HPP, which abates during the initial months of life. Approximately 10% of HE patients and all HPP patients have mild-to-moderate anemia with clinical features of pallor, jaundice, anemia, and gallstones. The most severe form of elliptocytosis, HPP, typically is inherited recessively and is characterized by a striking micropoikilospherocytosis and fragmentation with some elliptocytes. A markedly low MCV, typically in the range of 50 to 60 fL, may be observed. In HPP, RBCs are thermally unstable and fragment at temperatures of 46°C to 48°C, reflecting the presence of mutant spectrin in the cells. Additional specialized laboratory investigation includes separation of solubilized membrane proteins by polyacrylamide gel electrophoresis, which may reveal either an abnormally migrating spectrin or a deficiency or abnormal migration of protein 4.1. An increased fraction of unassembled dimeric spectrin can be detected by electrophoresis of RBC membrane extracts under nondenaturing conditions.

Treatment is not necessary for most individuals with common HE/HPP. Splenectomy may be of benefit for patients with symptomatic hemolytic anemia or its complications (see earlier discussion of splenectomy for hereditary spherocytosis).

KEY POINTS

- HE/HPP is due to defects in the interactions of red cell cytoskeleton proteins ("horizontal interactions"), with spectrin abnormalities accounting for most of the cases.
- The majority of patients with HE are not symptomatic and require no therapy.
- HPP is a severe form of HE with apparent coinheritance of spectrin defects leading to markedly abnormal red cells characterized by increased thermal instability.

Stomatocytosis

Stomatocytes have a wide transverse slit or stoma toward the center of the RBC (Figure 8-4). A few stomatocytes (between 3% and 5%) are found on blood smears of healthy individuals. Several inherited and acquired disorders are associated with stomatocytosis. The inherited forms are associated with abnormalities in erythrocyte

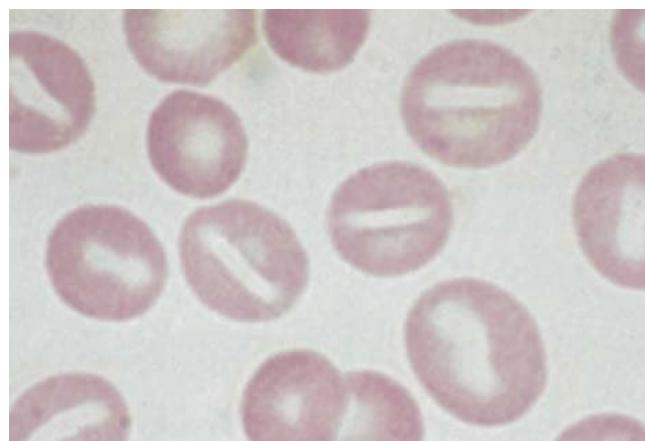


Figure 8-4 Stomatocytes.

cation permeability and volume, which is either increased (OHS), decreased (DHS or xerocytosis), or near normal. Like other hereditary membranopathies, genetic and clinical manifestations are highly heterogeneous. Hereditary stomatocytosis can be classified into syndromic and non-syndromic forms. Syndromic forms of hereditary stomatocytosis are rare, and they include (a) stomatin-deficient cryohydrocytosis with mental retardation, seizures, and hepatosplenomegaly, caused by mutations in the *SLC2A1* gene; (b) phytosterolemia nonleaky stomatocytosis with macrothrombocytopenia, where mutations in the *ABCG5* or *ABCG8* genes lead to increased absorption and decreased excretion of sterols, resulting in xanthelasmias, accelerated atherosclerosis, severe hypercholesterolemia, abnormal incorporation of sterols into RBC membrane; and (c) DHS with perinatal edema and/or pseudohyperkalemia due to mutations in the *PIEZ01* gene, a mechanosensitive cation channel. Mutations in *PIEZ01* and *KCNN4* genes can result in non-syndromic DHS, due to dehydration from abnormal RBC membrane permeability to Na⁺ and K⁺. Of significant clinical importance is the recognition that patients with *PIEZ01*-associated DHS have a very high risk of developing thrombotic events after splenectomy. Therefore, splenectomy is contraindicated. OHS can either be caused by the *RHAG* or *SLC4A1* gene, in which the cation leak result in excess intracellular water content (overhydrated) with microcytosis and low MCHC. Diagnosis of hereditary stomatocytosis is based on a combination of clinical presentation, macrocytic anemia and reticulocytosis, familial history with an autosomal dominant inheritance pattern, and laboratory testing. EMA test is almost normal in hereditary stomatocytosis and as mentioned previously, osmotic gradient ektacytometry can be useful in distinguishing hereditary

stomatocytosis from HS and confirmed via genetic testing. Targeted next-generation sequencing (NGS) may be useful in identifying the causal mutation. Acquired stomatocytosis can be seen in acute alcoholism and hepatobiliary disease (although target cells are more common) and occasionally in malignant neoplasms and cardiovascular disorders. Stomatocytes also may occur as an artifact.

Acanthocytosis

Spur cells, or acanthocytes (from the Greek *acantha*, or thorn; Figure 8-5), are erythrocytes with multiple irregular projections that vary in width, length, and surface distribution. Several conditions are associated with this morphology. In severe liver disease, acanthocyte formation is a 2-step process involving the transfer of free nonesterified cholesterol from abnormal plasma lipoproteins into the erythrocyte membrane and then the subsequent remodeling of abnormally shaped erythrocytes by the spleen. Rapidly progressive hemolytic anemia is seen in association with advanced and often end-stage alcoholic cirrhosis, sometimes referred to as Zieve syndrome, or other conditions such as metastatic liver disease, cardiac cirrhosis, Wilson disease, and fulminant hepatitis.

Abetalipoproteinemia

In abetalipoproteinemia, the primary molecular defect involves a congenital absence of apolipoprotein B in plasma. Consequently, all plasma lipoproteins containing this apoprotein as well as plasma triglycerides are nearly absent. Plasma cholesterol and phospholipid levels also are markedly reduced. The role of these lipid abnormalities in producing acanthocytes is not well understood. The

most striking abnormality of the acanthocyte membrane in abetalipoproteinemia is an increase in membrane sphingomyelin. Abetalipoproteinemia is an autosomal recessive disorder that manifests in the first month of life with steatorrhea. Retinitis pigmentosa and progressive neurologic abnormalities, such as ataxia and intention tremors, develop between 5 and 10 years of age and progress to death by the second or third decade of life. Therefore, it is crucial that patients are diagnosed promptly upon suspicion of the disease so that early treatment can be initiated to halt disease progression and recover normal neurological function. Treatment includes strict adherence to a low-fat diet, supplementation with essential fatty acids and fat-soluble vitamins. Patients also need to be monitored for ophthalmologic, neurologic, hematologic, and hepatic complications.

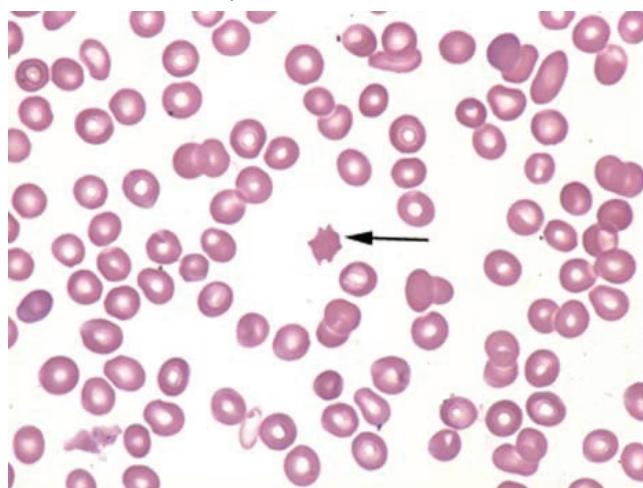
McLeod phenotype

Acanthocytes have also been described in patients with the McLeod phenotype, a condition in which the erythrocytes have reduced surface Kell antigen. The affected red cells lack the Kx protein which is a membrane precursor of the Kell antigen and needed for its expression. The Kx antigenic protein is encoded by the X chromosome, so males are affected with mild compensated hemolysis and variable acanthocytosis (8% to 85%). Due to lyonization, female carriers are asymptomatic with occasional acanthocytes and may be identified by flow cytometric analysis of Kell blood group antigen expression. In some ethnicities, the frequency of the Kx antigen is >99%, and thus, individuals with the McLeod phenotype can develop major problems with alloimmunization after immunizing events such as a transfusion. As such, autologous donation should be considered where possible. The McLeod phenotype is a key feature of McLeod syndrome, a rare multisystem disease characterized by neuropsychiatric, neuromuscular, cardiac, and hematological abnormalities. The subtle hematological abnormalities may precede the neurological complications for decades until patients develop premature dementia, cognitive impairment, social retraction, personality changes, and a choreatic movement disorder or dystonia. McLeod phenotype has also been associated with X-linked granulomatous disease.

Rh deficiency (null) syndrome

This term is used to designate rare cases of either absent (Rh_{null}) or markedly reduced (Rh_{mod}) expression of the Rh antigen in association with mild to moderate hemolytic anemia. Three proteins (RhCE, RhD, and Rh50) comprise the Rh protein family. This disorder arises through auto-

Figure 8-5 Acanthocytes.



somal recessive inheritance of either a suppressor gene unrelated to the Rh locus or a silent allele at the locus itself. The normal, complexed structure forms an integral membrane protein; its loss disrupts membrane architecture. Rh_{null} cells have increased rates of cation transport and sodium-potassium membrane adenosine triphosphate (ATP)-ase activity that results in dehydrated RBCs. This dehydration results in stomatocytes and occasional spherocytes on the peripheral blood smear. Laboratory evaluation shows increased RBC osmotic fragility, reflecting a marked reduction of the membrane surface area. The relationship between the absence of the Rh antigen proteins and RBC alterations leading to hemolysis presumably involves membrane microvesiculation, leading to diminished erythrocyte flexibility. Splenectomy results in improvement of the hemolytic anemia.

Abnormalities of RBC enzymes

CLINICAL CASE



A 23-year-old African American male who recently underwent cadaveric renal transplant for end-stage renal disease secondary to nephrotic syndrome is referred for urgent evaluation of anemia. His post-transplant course has been unremarkable with good graft function and no rejection. When he left the hospital, his hemoglobin was 103 g/L. His discharge medications included prednisone, cyclosporine, trimethoprim/sulfamethoxazole, and acyclovir. The day after discharge, he complains of acute onset of severe fatigue and dyspnea. Friends have noted yellowing of his eyes. He denies any fever or infectious symptoms. On physical examination, he has a heart rate of 112, blood pressure (BP) of 89/45, and scleral icterus. Otherwise, the examination is unremarkable. Current hemoglobin is 66 g/L, absolute reticulocyte count $477 \times 10^9/L$, LDH 1,543 U/L. Serum creatinine is 137 $\mu\text{mol}/L$ and the platelet count $302 \times 10^9/L$, similar to hospital discharge. On review of the peripheral blood smear, polychromatophilia is noted. A moderate number of bite and blister cells are identified.

Normal metabolism of the mature RBC involves 2 principal pathways of glucose catabolism: the glycolytic pathway and the hexose-monophosphate shunt. The 3 major functions of the products of glucose catabolism in the erythrocyte are (1) maintenance of protein integrity, cellular deformability, and RBC shape; (2) preservation of hemoglobin iron in the ferrous form; and (3) modulation of the oxygen affinity of hemoglobin. These functions are served by the regulation of appropriate production of 5 specific molecules: ATP, reduced glu-

tathione, reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), and 2,3-bisphosphoglyceric acid (BPG). Maintenance of the biochemical and structural integrity of the RBC depends on the normal function of >20 enzymes involved in these pathways as well as the availability of 5 essential RBC substrates: glucose, glutathione, NAD, NAD phosphate (NADP), and adenosine diphosphate.

The primary function of the glycolytic pathway is the generation of ATP, which is necessary for the ATPase-linked sodium-potassium and calcium membrane pumps essential for cation homeostasis and the maintenance of erythrocyte deformability. The production of 2,3-BPG in this pathway is regulated by the Rapoport-Luebering shunt, which is controlled by bisphosphoglyceromutase, the enzyme that converts 1,3-BPG to 2,3-BPG. Concentration of 2,3-BPG in the RBC in turn regulates hemoglobin oxygen affinity, thus facilitating the transfer of oxygen from hemoglobin to tissue-binding sites. The major function of the hexose-monophosphate shunt is preservation and regeneration of reduced glutathione, which protects hemoglobin and other intracellular and membrane proteins from oxidant injury.

Abnormalities of the glycolytic pathway

PK deficiency is the most common congenital nonspherocytic hemolytic anemia caused by a defect in glycolytic RBC metabolism. PK enzymes are a product of 2 distinct genes, *PKLR* (encoding the liver [L] and RBC [R] isoenzymes) and *PKM* (encoding the muscle [M] isoenzyme). PK deficiency is due to mutations of the *PKLR* gene located in chromosome 1q21. Rarely, mutations in the Kruppel-like factor 1 (*KLF-1*) gene have also been shown to reduce PK activity. It is autosomal recessively inherited and clinically heterogeneous. PK deficiency has a worldwide distribution. Presentation can range from jaundice, splenomegaly, and failure to thrive in the neonatal period or early childhood to a mild presentation with fully compensated hemolytic anemia. Patients with non-missense/non-missense mutations tend to be diagnosed earlier, with lower hemoglobin value, higher number of lifetime transfusions, and transfusion dependency compared to patients with non-missense/missense and missense/mis-sense mutations. Because 2,3-BPG level is increased in the RBCs, anemia from PK deficiency is better tolerated than anemias from other causes of hemolysis. Reference laboratories can perform quantitative measurement of the erythrocyte enzyme level necessary to diagnose this condition accurately. Reticulocytes have higher levels

of PK and extreme reticulocytosis, commonly seen after splenectomy, may result in borderline low or even normal PK activity. In such a situation, it is essential to compare the activity of PK relative to the other red cell enzymes such as glucose-6-phosphate dehydrogenase (G6PD) and hexokinase. A disproportionately "normal" PK level suggests PK deficiency.

Both glucose phosphate isomerase and hexokinase deficiencies produce nonspherocytic hemolytic anemia associated with decreased erythrocyte ATP and 2,3-BPG content. These disorders are rare; patients often present in childhood with mild to moderate anemia and reduced exercise tolerance. A form of acquired hexokinase deficiency occurs in Wilson disease, in which elevated copper levels in the blood inhibit hexokinase in a fluctuating fashion that may lead to intermittent brisk intravascular hemolysis. Phosphofructokinase deficiency was first described as a muscle glycogen storage disease; some patients with this deficiency have a chronic hemolytic anemia. In phosphofructokinase deficiency, low levels of erythrocyte ATP lead to low-grade hemolysis, but the limiting symptoms are usually weakness and muscle pain on exertion. Children with phosphoglycerate kinase have associated neuromuscular manifestations, including seizures, spasticity, and mental retardation.

These enzymopathies are associated with anemia of variable severity. Peripheral blood smears from patients with PK deficiency may show small dense crenated cells (echinocytes or "prickle cells"). However, the RBC morphology is frequently normal. In the most severe cases, marked reticulocytosis, nucleated RBCs, and substantial anisopoikilocytosis can be seen. The MCV is usually normal or increased, reflecting the contribution of reticulocytes. A marked increase in the reticulocyte count (up to 70%) occurs after splenectomy in PK deficiency.

Patients with severe hemolysis should receive folate supplementation. Splenectomy generally is reserved for patients with poor quality of life, chronic transfusion requirements, need for cholecystectomy, and persistent severe anemia. The response is variable, but most patients with PK deficiency benefit with an increase in the hemoglobin level. Splenectomy may be complicated by postoperative thromboembolic phenomena.

Abnormalities of the hexose-monophosphate shunt

G6PD is the major enzyme involved because deficiencies of the 2 downstream enzymes 6-phosphogluconolactonase and 6-phosphogluconate dehydrogenase are extremely rare and not always associated with hemolysis.

G6PD deficiency

G6PD deficiency is the most frequently encountered abnormality of RBC metabolism, affecting >400 million people worldwide. A survival advantage has been noted in G6PD-deficient patients infected with *P. falciparum* malaria, possibly accounting for its high gene frequency, especially in endemic regions. However, this concept was recently challenged by the results of a large genetic study.

The gene for G6PD is carried on the X chromosome and exhibits extensive polymorphism. Enzyme deficiency is observed in males carrying a variant gene. Females with a variant gene have 2 RBC populations, 1 normal and 1 deficient; the clinical presentation depends on the extent of inactivation ("Lyonization") of the affected X chromosome bearing the abnormal gene. Worldwide, >300 genetic variants of G6PD have been described and are categorized by the World Health Organization according to the extent of enzyme deficiency and severity of hemolysis: class I (chronic non-spherocytic hemolytic anemia), class II (<10% activity and intermittent hemolysis), class III (10% to 60% activity and intermittent hemolysis), class IV (normal activity and no hemolysis), and class V (increased activity). G6PD enzyme variants are distinguished based on electrophoretic mobility. G6PD B, the wild-type enzyme, and G6PD A⁺, a common variant in the African American population, demonstrate normal enzyme activity and are not associated with hemolysis. G6PD A⁻ is present in approximately 10% to 15% of African American males. This variant is an unstable enzyme, which results in a decrease in enzyme activity in aged RBCs. Hemolysis is typically self-limited. In contrast, other G6PD variants have reduced catalytic activity and marked instability or are produced at a decreased rate, rendering both reticulocytes and older cells susceptible to hemolysis. Enzymatic deficiency of this type is seen in up to 5% of persons of Mediterranean or Asian ancestry, as well as Ashkenazi Jews. The common example of this deficiency is G6PD-Mediterranean.

Pathophysiology. Hemolysis in G6PD-deficient RBCs is due to a failure to generate adequate NADPH, leading to decreased ratio of reduced to oxidized glutathione. This renders erythrocytes susceptible to oxidation of hemoglobin by oxidant radicals, such as hydrogen peroxide. The resulting denatured hemoglobin aggregates and forms intraerythrocytic Heinz bodies, which bind to membrane cytoskeletal proteins. Membrane proteins are also subject to oxidation, leading to decreased cellular deformability. Cells containing Heinz bodies are entrapped or partially

destroyed in the spleen, resulting in loss of cell membranes through pitting of Heinz bodies and leading to hemolysis. Of note, Heinz bodies are not specific to G6PD and can be seen in thalassemias and unstable hemoglobinopathies. If the oxidant stress is severe, intravascular hemolysis may occur.

The severity of hemolytic anemia in patients with G6PD deficiency depends on the type of defect, the level of enzyme activity in the erythrocytes, and the severity of the oxidant challenge. Ingestion of an oxidant drug or fava beans is sometimes the precipitating cause (Table 8-3). Some drugs have been confirmed to cause hemolysis in G6PD-deficient individuals, while for others no firm evidence exists to implicate their association and disagreements exist between literature sources. Hemolytic anemia in patients with G6PD deficiency may first be recognized during an acute clinical event that induces oxidant stress, such as infection, diabetic ketoacidosis, or severe liver injury. In children, infection is a common precipitating event. Individuals with G6PD A⁻ do not manifest anemia until they are exposed to an oxidant drug or other oxidant challenge. Such an exposure may provoke an acute hemolytic episode with intravascular hemolysis. In the G6PD A⁻ variant, an adequate reticulocyte response can result in restoration of the hemoglobin concentration even if the offending drug is continued because the newly formed reticulocytes are relatively resistant to oxidant stress given their higher G6PD levels. Women heterozygous for G6PD A⁻ usually experience only mild anemia upon exposure to oxidant stress because a pop-

ulation of G6PD-sufficient (normal) cells coexists. The G6PD-Mediterranean variant is more severe than the African G6PD A⁻ variant and is thus prone to more severe hemolytic episodes. Men and heterozygous women with the G6PD-Mediterranean variant can experience severe hemolysis in the face of oxidant stress, and the offending agent must be removed because the reticulocytes have low enzyme levels and are prone to hemolysis. Fava beans can trigger severe hemolysis, a condition termed “favism.”

Clinical manifestations, laboratory evaluation, and treatment. The most common presentation is acute hemolysis provoked by oxidant drug or illness. Favism is less common except in southern Europe, Middle East, and Southeast Asia, where fava beans are a popular food. However, hemolytic anemia due to favism may be severe or even fatal, particularly in children. G6PD deficiency predisposes to neonatal jaundice, and it may be the result of impairment of hepatic function, hemolysis, or both. Certain rare G6PD variants may result in a chronic nonspherocytic hemolytic anemia with persistent splenomegaly.

G6PD deficiency should be considered in an individual with evidence of chronic DAT-negative hemolysis. The peripheral blood smear may show RBCs with the hemoglobin confined to one side of the cells, with the remainder appearing as a hemoglobin-free ghost (eccentrocytes) (Figure 8-6). The morphology previously has been described as bite or blister cells, interpreted as the result of

Table 8-3 Drugs that can cause clinically significant hemolysis in patients with G6PD deficiency

Drug category	Predictable hemolysis	Possible hemolysis
Antimalarials	Dapsone Primaquine	Chloroquine Quinine
Analgesics/antipyretics	Phenazopyridine	Aspirin (high dose) Acetaminophen (paracetamol)
Antibacterials	Trimethoprim-sulfamethoxazole Sulfadiazine Quinolones (nalidixic acid, ciprofloxacin, ofloxacin) Nitrofurantoin	Chloramphenicol Isoniazid Sulfasalazine
Other	Methylene blue Rasburicase Toluidine blue	Ascorbic acid Glibenclamide Isosorbide dinitrate Vitamin K

Adapted from Luzzatto L, Seneca E, *Br J Haematol*. 2014;164(4):469–480.

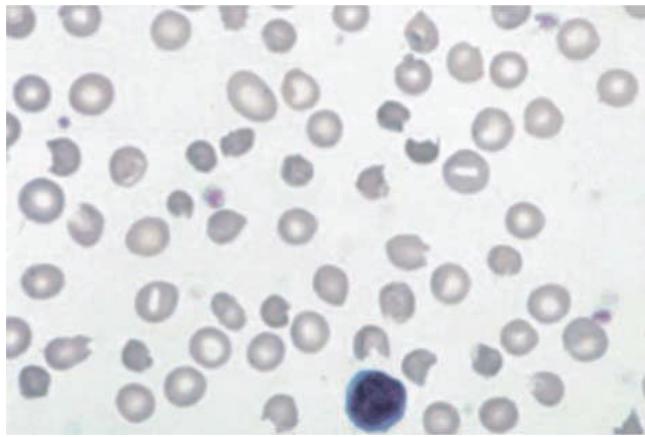


Figure 8-6 G6PD deficiency. The peripheral blood smear shows several red cells with the hemoglobin confined to one side of the cells, with the remainder appearing as a hemoglobin-free ghost (eccentrocytes).

removal of denatured hemoglobin by the spleen; however, it appears that the accumulated oxidized hemoglobin actually remains and is adherent to the RBC membrane. Brilliant cresyl blue staining may reveal Heinz bodies. Screening or quantitative biochemical assays can be used to make the diagnosis. In the G6PD A⁻ variant, during an acute hemolytic episode, an elevated reticulocyte count raises the mean level of erythrocyte G6PD and render a false-negative result. G6PD levels, therefore, should be checked several months after the acute event when there will be RBCs of varying ages. As previously discussed in PK deficiency, comparative assessment of the activity levels of unaffected RBC enzymes may help in the test interpretation during an acute episode.

Management is primarily avoidance or discontinuation of oxidant drugs and avoidance of fava beans. When anemia is severe, RBC transfusion may be necessary.

Abnormalities of nucleotide metabolism

Pyrimidine-5'-nucleotidase deficiency is an enzymatic abnormality of pyrimidine metabolism associated with hemolytic anemia. The peripheral blood smear in patients with this defect often shows RBCs containing coarse basophilic stippling. Lead intoxication also inactivates the enzyme, leading to an acquired variant of pyrimidine-5'-nucleotidase deficiency.

Adenosine deaminase (ADA) excess is an unusual abnormality and is the only red cell enzyme disorder that is inherited in an autosomal dominant manner, but the molecular mechanism of this disorder has not been identified. It is caused by a genetically determined increase in the

activity of a normal erythrocyte enzyme. The excessive deaminase activity prevents normal salvage of adenosine and causes subsequent depletion of ATP and hemolysis. Deficiency of ADA is associated with severe combined immunodeficiency.

CLINICAL CASE (continued)

The patient presented in this section should be suspected of having G6PD deficiency. Patients with the African American variant (G6PD A⁻) are often asymptomatic until they ingest medication or experience an infection, which leads to oxidant stress of the RBCs. Trimethoprim/sulfamethoxazole may be an offending agent. During the early phases of hemolysis, eccentrocytes can be seen on review of the peripheral blood smear. A Heinz body preparation may show the typical inclusions, which consist of denatured hemoglobin. G6PD levels may be misleading in the acute setting, as values may be normal due to reticulocytosis. Treatment is primarily supportive. Offending drugs should be discontinued and alternative agents chosen. If the prescribed agent is necessary and cannot be substituted, a trial of continuation is reasonable, as hemolysis often is compensated in the G6PD A⁻ variant even if drug administration is continued.

Establishing the genetic causes in patients with intrinsic abnormalities of the RBC

As indicated above, intrinsic abnormalities of the RBC are phenotypically diverse and genetically heterogeneous. Extensive biochemical work-up may be inconclusive and multiple rounds of single gene testing are costly to perform. Various NGS panels have been developed independently in the past decade that allow high throughput sequencing of multiple candidate genes in parallel. Targeted NGS is the approach chosen most often in determining the molecular basis of congenital hemolytic disorders. Targeted NGS involves sequencing genes known to cause congenital hemolytic disorders. Targeted NGS is especially helpful in severe cases that are heavily transfused and therefore functional biochemical studies cannot be performed, or in cases where the amount of blood required for biochemical testing is too large (eg, enzyme assays in neonates). Genes chosen vary from panel to panel. The average number of times the coding region, splice site junctions, intronic and regulatory regions are sequenced to improve accuracy (known as coverage) also varies between panels. Costs, accuracy, time, and read length vary between NGS plat-

forms. As such, failure to determine the causal variant by targeted NGS does not denote the absence of disease or a genetic cause. In these cases, there may be a need to proceed to whole exome sequencing (WES) or genome sequencing (WGS) to elucidate the existence of a new causal variant or new causal genes. Even then, WES and WGS are not very sensitive methods to detect long insertion-deletion, copy-number, structural, and epigenetic variants. Therefore, targeted NGS is not useful for “ruling out” a genetic cause in congenital hemolytic disorder. The clinical significance of isolated variants should be established via clinical history, physical examination, laboratory tests, family history, along with previous reports of known pathogenicity, to determine penetrance and mode of inheritance. Sometimes further laboratory testing and experimentation are required to determine the pathogenicity of the variant in question. This is especially true in cases where the clinical presentation is atypical. Such cases are usually referred to research or reference centers for further investigations. A guideline for variant interpretation has been established by the American College of Medical Genetics and Genomics.

KEY POINTS

- The glycolytic pathway generates ATP, which is necessary for maintenance of RBC membrane integrity and oxygen affinity.
- Glucose metabolism through the hexose monophosphate shunt produces NADPH to maintain the antioxidative activity of the RBC.
- Enzymopathies represent a major consideration in the differential diagnosis of inherited DAT-negative nonspherocytic hemolytic anemias.
- PK deficiency is the most common defect of the glycolytic pathway and G6PD deficiency, the most common defect of the hexose monophosphate shunt.
- In G6PD deficiency, quantitative measurement of the enzyme levels during an acute hemolytic episode may be falsely elevated.
- Defects of purine and pyrimidine metabolism are infrequent. The peripheral blood smear in pyrimidine-5'-nucleotidase deficiency shows red cells with coarse basophilic stippling.
- Iron overload can occur in nontransfused inherited chronic hemolytic anemias due to increased gastrointestinal iron absorption.

Hemolysis due to extrinsic abnormalities of the RBC

CLINICAL CASE

A 68-year-old male is admitted to the hospital with complaints of weakness, shortness of breath, and chest pain. Over the prior year, he has experienced weight loss and intermittent night sweats, and has generally felt poorly. His prior history is significant for diet-controlled diabetes and elevated cholesterol. He is taking no medications. On examination, he appears chronically ill and pale. Scleral icterus is noted. Axillary adenopathy and splenomegaly are appreciated. His fingertips appear mildly cyanotic. Laboratory data are significant for a hemoglobin of 84 g/L and an MCV of 143 fL. LDH is elevated at 2,321 U/L, indirect bilirubin 36 µmol/L, and absolute reticulocyte count $301 \times 10^9/L$. The peripheral blood smear shows agglutinated RBCs. The blood bank reports DAT positive for complement (3+) but negative for immunoglobulin G (IgG). Serum protein electrophoresis reveals a monoclonal IgMk. Abdominal CT scan reveals splenomegaly and diffuse adenopathy.

Hemolytic anemia due to immune injury to RBCs

In autoimmune hemolytic anemia (AHA), shortened RBC survival is mediated by autoantibodies. AHA is classified by the temperature at which autoantibodies bind optimally to the patient RBCs. In adults, the majority of cases (80% to 90%) are mediated by antibodies that bind to RBCs at 37°C (warm autoantibodies). In the cryopathic hemolytic anemias, the autoantibodies bind most avidly to RBCs at temperatures <37°C (cold autoantibodies). Some patients exhibit both warm and cold reactive autoantibodies. These cases are classified as mixed AHA.

The warm- and cold-antibody classifications are further divided by the presence or absence of an underlying related disease. When no underlying disease is recognized, the AHA is termed *primary* or *idiopathic*. *Secondary* cases are those in which the AHA is a manifestation or complication of an underlying disorder. In general, the secondary classification should be used in preference to idiopathic only when the AHA and the underlying disease occur together more often than randomly and when the AHA resolves with successful treatment of the underlying disease. The connection is strengthened when the underlying disease has a component of immunologic aberration. Using these criteria, primary (idiopathic) AHA and secondary AHA occur with approximately equal frequency.

Certain drugs also may cause immune destruction of RBCs by 3 different mechanisms. Some drugs induce

Table 8-4 Classification of immune injury to red blood cells

I. Warm-autoantibody type: autoantibody maximally active at 37°C	
A. Primary or idiopathic warm AHA	
B. Secondary warm AHA	
1. Associated with lymphoproliferative disorders (eg, chronic lymphocytic leukemia, non-Hodgkin lymphoma)	
2. Associated with the rheumatic disorders (eg, SLE)	
3. Associated with certain nonlymphoid neoplasms (eg, ovarian tumors)	
4. Associated with certain chronic inflammatory diseases (eg, ulcerative colitis)	
5. Associated with certain drugs (eg, cephalosporins, NSAIDs)	
II. Cold-autoantibody type: autoantibody optimally active at temperatures <37°C	
A. Mediated by cold agglutinins	
1. Idiopathic (primary) chronic cold agglutinin disease (usually associated with IgMκ monoclonal gammopathy of undetermined significance)	
2. Secondary cold agglutinin hemolytic anemia	
a. Postinfectious (eg, <i>Mycoplasma pneumoniae</i> or infectious mononucleosis)	
b. Associated with malignant B-cell lymphoproliferative disorder	
B. Mediated by cold hemolysins	
1. Idiopathic (primary) paroxysmal cold hemoglobinuria	
2. Secondary paroxysmal cold hemoglobinuria	
a. Associated with an acute viral syndrome in children	
b. Associated with congenital or tertiary syphilis in adults	
III. Mixed cold and warm autoantibodies	
A. Primary or idiopathic mixed AHA	
B. Secondary mixed AHA	
1. Associated with the rheumatic disorders, particularly SLE	
IV. Drug-immune hemolytic anemia	
A. Hapten or drug adsorption mechanism	
B. Ternary (immune) complex mechanism	
C. True autoantibody mechanism	

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SLE, systemic lupus erythematosus; NSAIDs, nonsteroidal anti-inflammatory drugs.

formation of true autoantibodies directed against RBC antigens. The hapten-drug adsorption mechanism is characterized by the presence of antidrug antibodies in the blood. These antibodies bind only to RBC membranes that are coated with tightly bound drug. In a third type of drug-immune hemolytic anemia, antibodies recognize a neoantigen formed by a drug or its metabolite and an epitope of a specific membrane antigen. This is termed *ternary* or *immune complex mechanism*. In some (if not all) cases mediated by the ternary (immune) complex mechanism, antibodies may recognize both a drug or its metabolite and an epitope of a specific RBC antigen. The classification of the immune hemolytic anemias is shown in Table 8-4.

Pathophysiology

Warm AHA

The most common type of AHA is mediated by warm-reactive autoantibodies of the IgG isotype. Warm-reacting IgG antibodies bind optimally to antigens on RBCs at 37°C and may or may not fix complement, but they typically do not cause direct agglutination of RBCs because of their small and monomeric conformation. Enhanced destruction of antibody-coated RBCs is mediated by Fc receptor-expressing macrophages, primarily located in the spleen. Partial phagocytosis results in the formation of spherocytes that may circulate for a time but eventually become entrapped in the spleen, resulting in enhanced RBC destruction.

Cold AHA

In contrast to warm-reactive autoantibodies, cold-reactive autoantibodies bind optimally to RBCs at temperatures <37°C. Cold autoantibodies are typically of the IgM isotype, and because of their large and pentameric conformation, they are able to span the distance between several RBCs to cause direct agglutination. Their ability to injure RBCs depends on their ability to fix complement. The consequence of complement fixation is clearance of C3d-coated cells by attachment to complement receptors on macrophages, primarily in the spleen, and Kupffer cells in the liver. Direct lysis by completion of the terminal complement sequence may also occur. Cold autoantibodies are characteristic of AHA associated with *Mycoplasma* infection, as well as with Epstein-Barr virus-related disease. In addition, cold agglutinin disease (CAD) is typically seen in the elderly, almost always associated with B-cell lymphoproliferative disorders, especially monoclonal gammopathy of undetermined significance. It is caused by a monoclonal IgM antibody that binds to carbohydrate I antigens or i antigens at temperatures below body temperature. Cold-reacting IgG (Donath-Landsteiner) autoantibodies, seen in paroxysmal cold hemoglobinuria (PCH), may cause significant intravascular lysis of RBCs as a result of their ability to fix complement. PCH frequently was associated with congenital syphilis in the past. Now, it is almost always idiopathic. PCH accounts for ~10% of AHA cases in children. The responsible autoantibodies bind to antigens in the P blood group system.

Mixed AHA

Some cases of AHA are associated with the presence of both IgM and IgG autoantibodies. Hemolysis is generally more severe in these cases. AHA due to IgA antibodies is rare. IgA autoantibodies usually are accompanied by IgG autoantibodies. The mechanisms for RBC destruction appear to be similar to those for IgG.

Drug-induced immune hemolytic anemia

The clinical and laboratory features of drug-induced and idiopathic hemolytic anemia are similar, so a careful history of drug exposure should be obtained in the initial evaluation. The number of drugs that can cause immune hemolytic anemia is large and encompasses a broad spectrum of chemical classes (Table 8-5). Three basic mechanisms of drug-induced immune RBC injury are recognized. A fourth mechanism may lead to nonimmunologic deposition on RBCs of multiple serum proteins, including immunoglobulins, albumin, fibrinogen, and others; but RBC injury does not occur. The mechanisms of drug-induced immune hemolytic anemia and positive DATs are summarized in

Table 8-5 Drugs associated with immune injury to RBCs or a positive direct antiglobulin test

Hapten or drug adsorption mechanism	
Carbromal	Oxaliplatin
Cephalosporins	Penicillins
Cianidanol	Tetracycline
Hydrocortisone	Tolbutamide
6-Mercaptopurine	
Ternary-immune complex mechanism	
Amphotericin B	Nomifensine
Antazoline	Oxaliplatin
Cephalosporins	Pemetrexed
Chlorpropamide	Probencid
Diclofenac	Quinime
Diethylstilbestrol	Quinidine
Doxepin	Rifampicin
Etodolac	Stibophen
Hydrocortisone	Thiopental
Metformin	Tolmetin
Autoantibody mechanism	
Cephalosporins	Lenalidomide
Cianidanol	Mefenamic acid
Cladribine	α-Methyldopa
Diclofenac	Nomifensine
L-DOPA (levodopa)	Oxaliplatin
Efalizumab	Pentostatin
Fludarabine	Procainamide
Glafenine	Teniposide
Latamoxef	Tolmetin
Nonimmunologic protein adsorption	
Carboplatin	Cisplatin
Cephalosporins	Oxaliplatin
Uncertain mechanism of immune injury	
Acetaminophen	Melphalan
p-Aminosalicylic acid	Mephenytoin
Carboplatin	Nalidixic acid
Chlorpromazine	Omeprazole
Efavirenz	Phenacetin
Erythromycin	Streptomycin
Fluorouracil	Sulindac
Ibuprofen	Temafloxacin
Insecticides	Thiazides
Isoniazid	Triamterene

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Table 8-6 Immune hemolytic anemia and positive direct antiglobulin reactions caused by drugs

	Hapten-drug adsorption	Ternary-immune complex formation	Autoantibody formation	Nonimmunologic protein adsorption
Prototype drug	Penicillin	Third-generation cephalosporins	α -Methyldopa	Cephalothin
Role of drug	Binds to red cell membrane	Forms 3-way complex with antibody and red cell membrane component	Induces antibody to native red cell antigen	Possibly alters red cell membrane
Drug affinity to cell	Strong	Weak	None demonstrated	Strong
Antibody to drug	Present	Present	Absent	Absent
Antibody class predominating	IgG	IgM or IgG	IgG	None
Proteins detected by direct antiglobulin test	IgG, rarely complement	Complement	IgG, rarely complement	Multiple plasma proteins
Dose of drug associated with positive antiglobulin test	High	Low	High	High
Mechanism of red cell destruction	Splenic sequestration	Direct lysis by complement plus splenic sequestration	Splenic sequestration	None

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Ig, immunoglobulin.

Table 8-6. In recent series, cephalosporins, penicillin derivatives, and nonsteroidal anti-inflammatory drugs account for >80% of drug-induced immune hemolytic anemia.

Hapten or drug adsorption mechanism

Hapten or drug adsorption mechanism applies to drugs that bind firmly to proteins on the RBC membrane. The classic setting is very-high-dose penicillin therapy, but other drugs such as cephalosporins and semisynthetic penicillins also are implicated. The antibody responsible for hemolytic anemia by this mechanism is of the IgG class and is directed against epitopes of the drug. Other manifestations of drug sensitivity, such as hives or anaphylaxis, usually are not present. The antibody binds to drug molecules attached to the RBC membrane. Antibodies eluted from patients' RBCs or present in their sera react in the indirect antiglobulin test only against drug-coated RBCs, which distinguishes these drug-dependent antibodies from true autoantibodies. Destruction of RBCs coated with drug and IgG antidrug antibody occurs mainly through sequestration by splenic macrophages. Hemolytic anemia typically occurs 7 to 10 days after the drug is started and ceases a few days to 2 weeks after the patient discontinues taking the drug.

Ternary or immune complex mechanism: drug antibody-target cell interaction

Drugs in this group exhibit only weak direct binding to blood cell membranes. A relatively small dose of drug is capable of triggering destruction of blood cells. Blood cell

injury is mediated by a cooperative interaction among 3 reactants to generate a ternary complex consisting of the drug or a drug metabolite, a drug-binding membrane site (an antigen) on the target cell, and a drug-dependent antibody. The drug-dependent antibody is thought to bind, through its Fab domain, to a compound neoantigen consisting of loosely bound drug and a blood group antigen intrinsic to the RBC membrane. The pathogenic antibody recognizes the drug only in combination with a particular membrane structure of the RBC (eg, a known alloantigen). Binding of the drug to the target cell membrane is weak until the attachment of the antibody to *both* drug and cell membrane is stabilized. Yet the binding of the antibody is drug dependent. RBC destruction occurs intravascularly after completion of the whole complement sequence, often resulting in hemoglobinemia and hemoglobinuria. The DAT is positive usually only for complement.

Autoantibody mechanism

Several drugs, by unknown mechanisms, induce the formation of autoantibodies reactive with RBCs in the absence of the instigating drug. The most studied drug in this category has been α -methyldopa, but levodopa and other drugs also have been implicated. Patients with chronic lymphocytic leukemia treated with pentostatin, fludarabine, or cladribine may have severe and sometimes fatal autoimmune hemolysis, although the mechanisms of autoantibody induction are likely different, most likely involving dysregulation of T lymphocytes.

Nonimmunologic protein adsorption

A small proportion (<5%) of patients receiving cephalosporin antibiotics, carboplatin, and cisplatin develop positive DAT because of nonspecific adsorption of plasma proteins to their RBC membranes. This process may occur within 1 to 2 days after the drug is instituted. Multiple plasma proteins, including immunoglobulins, complement, albumin, fibrinogen, and others, may be detected on RBC membranes in such cases. Hemolytic anemia resulting from this mechanism does not occur. This phenomenon, however, may complicate crossmatch procedures unless the drug history is considered.

Clinical manifestations and laboratory findings

Several clinical features of AHA are common to both warm- and cold-antibody types. Patients may present with signs and symptoms of anemia (eg, weakness, dizziness), jaundice, abdominal pain, and fever. Mild splenomegaly is common. Hepatomegaly and lymphadenopathy may be evident at presentation depending on the etiology. Anemia may vary from mild to severe, usually with either normocytic or macrocytic cells. Patients most frequently present with reticulocytosis. Reticulocytopenia, however, initially may be present up to one-third of the time as a result of intercurrent folate deficiency, infection, involvement of the marrow by a neoplastic process, or unidentifiable causes. Indirect bilirubin and LDH are elevated to varying degrees, and the haptoglobin is depressed. The blood smear often demonstrates spherocytes (Figure 8-7). Nucleated RBCs also may be present.

The onset of warm-antibody AHA may be rapid or insidious, but rarely is it so severe as to cause hemoglobinuria. Presenting symptoms usually are related to anemia or jaundice. In secondary cases, the presenting complaint usually is related to the underlying disease.

Patients with idiopathic or primary CAD usually have mild to moderate chronic hemolysis. Acute exacerbations can be associated with cold exposure. Spontaneous autoagglutination of RBCs at room temperature may be seen as clumps of cells on the blood smear (Figure 8-8). Occasionally, spurious marked elevations in the MCV and MCHC measurements and decrease in the RBC count are observed due to simultaneous passage of 2 or 3 agglutinated RBCs through the aperture of the automated cell counter.

Drug-immune hemolytic anemia due to the hapten or true autoantibody mechanism is usually mild. In contrast, hemolysis due to the ternary or immune complex mechanism can be acute in onset, severe, and sometimes fatal.

The DAT is usually positive in AHA but may be negative in some patients. The threshold of detection of commercial antiglobulin reagents, which detect mainly IgG and frag-

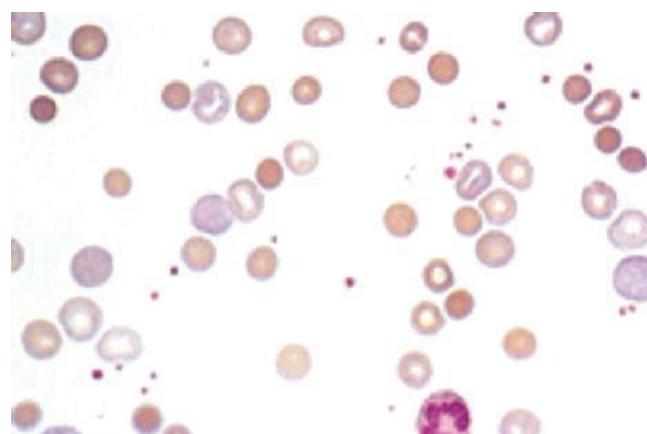


Figure 8-7 Warm-antibody autoimmune hemolytic anemia. Note the small round spherocytes and the large, gray polychromatophilic erythrocytes.

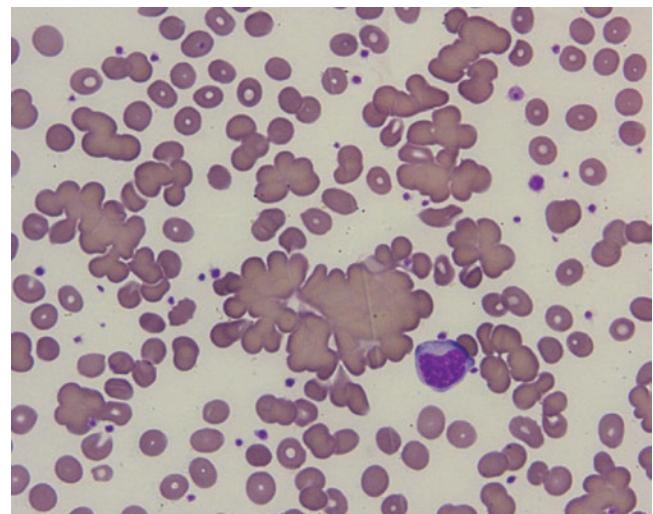


Figure 8-8 Cold agglutinin disease. Source: ASH Image Bank/John Lazarchick (image 00001053).

ments of C3, is approximately 200 to 500 antibody molecules per cell. However, <100 molecules of IgG per cell may significantly shorten RBC survival *in vivo*. IgM cold agglutinins are usually removed from RBCs during washing and usually are not detected. Most commercial reagents do not detect IgA. When monospecific anti-IgG and anti-C3 reagents are used, 30% to 40% of patients with AHA have only IgG on their RBCs; a slightly larger number have both IgG and C3; and only approximately 10% have C3 alone. The major reaction patterns of the DAT and their differential diagnosis are summarized in Table 8-7.

The strength of the DAT has poor clinical correlation with severity of hemolysis among patients, but in a given patient over time, the degree of hemolysis correlates fairly

Table 8-7 Differential diagnosis of reaction patterns of the direct antiglobulin test

Reaction pattern	Differential diagnosis
IgG alone	Warm antibody autoimmune hemolytic anemia
	Drug-immune hemolytic anemia: hapten/drug adsorption type or autoantibody type
Complement alone	Warm antibody autoimmune hemolytic anemia with subthreshold IgG deposition
	Cold-agglutinin disease
	Paroxysmal cold hemoglobinuria
	Drug-immune hemolytic anemia: ternary-immune complex type
IgG plus complement	Warm antibody autoimmune hemolytic anemia
	Drug-immune hemolytic anemia: autoantibody type (rare)

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well with the current strength of the antiglobulin reaction. In the rare case of DAT-negative hemolytic anemia suspected of having an immune etiology, the diagnosis sometimes can be confirmed by using more sensitive assays for RBC-bound immunoglobulin, such as an enzyme-linked immunoabsorbent assay (ELISA) or radiolabeled anti-immunoglobulin. Specific assays for cell-bound IgA also may be worthwhile. In CAD, the DAT is positive with anti-C3 only.

Approximately 1 in 10,000 healthy blood donors have a positive DAT. The positive DAT in these individuals usually is due to warm-reacting IgG autoantibodies, indistinguishable from those occurring in AHA. Many of these individuals never develop AHA, but some do. It is not known how many of these normal individuals with a positive DAT eventually may develop AHA.

Treatment

Asymptomatic patients develop anemia over a period sufficient to allow for cardiovascular compensation and do not require RBC transfusions. For patients with symptomatic coronary artery disease or patients who rapidly develop severe anemia with circulatory failure, as in PCH or ternary (immune) complex drug-immune hemolysis, transfusions can be lifesaving.

Transfusion of RBCs in immune hemolytic anemia is often problematic. Finding serocompatible donor blood is rarely possible because, in most cases, the autoantibody is a panagglutinin. It is most important to identify the patient's ABO type to find either ABO-identical or ABO-compatible blood for transfusion to avoid a hemolytic transfusion reac-

tion. The difficult technical issue relates to detection of RBC alloantibodies masked by the presence of the autoantibody.

Clinicians and blood bank physicians speak of identifying “least incompatible” blood for transfusion, but this is a misnomer because all units are serologically incompatible. Units incompatible because of autoantibody are less dangerous to transfuse, however, than units incompatible because of alloantibody. Patients with a history of pregnancy, abortion, or prior transfusion are at risk of harboring an alloantibody. Patients who have never been pregnant or transfused with blood products are unlikely to harbor an alloantibody. Consultation between the clinician and the blood bank physician should occur early to allow for informed discussion and confident transfusion of mismatched blood if the situation demands. Clinicians must understand that the dropping hemoglobin often seen in the setting of reticulocytopenia is a life-threatening situation, and delay in transfusion over concerns about red cell incompatibility can lead to a patient’s demise.

The selected RBCs should be transfused slowly while the patient is monitored carefully for signs of a hemolytic transfusion reaction. Even if transfused cells are rapidly destroyed, the increased oxygen-carrying capacity provided by the transfused cells may maintain the patient during the time required for other modes of therapy to become effective.

Warm AHA

In AHA, therapy is aimed at decreasing the production of autoantibodies and at decreasing clearance of RBCs from the circulation. For warm antibody IgG-mediated hemolysis, glucocorticoids such as prednisone usually are the first-line treatment in all but drug-induced syndromes (for which removal of the offending agent is the principal treatment). Glucocorticoids decrease the ability of macrophages to clear IgG- or complement-coated erythrocytes and reduce autoantibody production. After remission is achieved with prednisone at approximately 60 to 100 mg/d (or 1 mg/kg/d), the dose may be decreased by 20 mg/d each week until a dose of 20 mg/d is reached. Subsequent dose reduction should then proceed more slowly (at 5 mg/d per week), with the goal of either maintaining remission with prednisone at 20 to 40 mg every other day or complete weaning of prednisone if the DAT becomes negative; this goal is not always achievable. Approximately two-thirds of adult patients respond to prednisone, with about 50% achieving complete remission. Pulses of high-dose glucocorticoids (eg, 1 g methylprednisolone intravenously) are effective in some patients in whom standard therapy has failed.

Splenectomy is often considered if hemolysis remains severe for 2 to 3 weeks at prednisone doses of 1 mg/kg, if remission cannot be maintained on low doses of pred-

nisone, or if the patient has intolerable adverse effects or contraindications to glucocorticoids. It results in a reduced rate of clearance of IgG-coated cells. Although not usually recommended in children, splenectomy in patients past adolescence appears relatively safe. Patients should receive pneumococcal, *H. influenzae*, and meningococcal vaccines before splenectomy. Approximately two-thirds of patients have complete or partial remission with splenectomy, but relapses are common (40%).

Other therapies may be effective for patients with refractory hemolysis or for those who relapse after glucocorticoids or splenectomy. Standard-dose (375 mg/m^2) and low-dose (100 mg/m^2) rituximab is useful in refractory cases and is increasingly used prior to splenectomy. The response rates range from 70% to 90%, with long term relapse rates of approximately 50%. Immunosuppressive drugs, such as cyclophosphamide, azathioprine, mycophenolate mofetil, and cyclosporine, as well as the nonvirilizing androgen danazol have been used with varying degrees of success. Intravenous immunoglobulin has been less successful in treatment of AHA than in immune thrombocytopenic purpura.

Cold agglutinin disease

For patients with idiopathic CAD, maintaining a warm environment may be all that is needed to avoid symptomatic anemia. CAD responds to glucocorticoids less commonly (30%) than warm AHA and the duration of response is frequently short. Rituximab is the standard treatment regardless of whether CAD is associated with an IgM monoclonal gammopathy or not, and the response rate is about 50%. Chlorambucil and cyclophosphamide have been beneficial in selected cases. Rituximab in combination with chemotherapy (bendamustine, fludarabine, or prednisone) may be indicated if the disorder is associated with a lymphoproliferative disorder and if there is no response to rituximab. Splenectomy usually is not indicated because cells typically are cleared by intravascular hemolysis or hepatic Kupffer cells. Intravenous immunoglobulin does not have a role in management. Plasmapheresis may be temporarily effective in acute situations by removing IgM cold agglutinin from the circulation.

AHA during childhood tends to occur suddenly, during or after an acute infection. As many as one-third of cases are associated with intravascular hemolysis because of a Donath-Landsteiner antibody directed against the erythrocyte P antigen. Usually these patients exhibit only a single paroxysm of hemolysis. In warm AHA, acute management is similar to that for adults. Approximately two-thirds of children recover completely within a matter of weeks. Only a small percentage of children (but a larger proportion of adolescents) exhibit more chronic refrac-

tory disease that warrants consideration of other pharmacologic agents or splenectomy.

CLINICAL CASE (continued)

The patient presented in this section has CAD, likely secondary to underlying lymphoma. Automated techniques reveal the red cell count is artificially low, and the MCV and MCHC are falsely elevated secondary to red cell agglutination. Warming of the blood tube with immediate measurement and slide preparation minimizes agglutination. The DAT is positive only for complement. Lymphoproliferative disorders are well-identified underlying etiologies. The patient should be maintained in a warm environment. Amelioration of the anemia can be anticipated with cytotoxic therapy for the lymphoma.

KEY POINTS

- Warm-antibody-induced immune hemolytic anemia is typically IgG mediated and results in spherocytic red cells.
- CAD is IgM mediated with associated complement activation. The peripheral blood smear reveals red cell agglutination and spherocytes.
- A variety of drugs cause immune hemolytic anemia. Clinical laboratory support of the diagnosis may not be available. Discontinuation of the suspected offending drug is indicated.
- Symptoms resulting from AHA are typically indistinguishable from other causes of hemolysis.
- The DAT is the primary tool for diagnosing AHA. It is rarely positive in healthy individuals and may be negative in AHA.
- Warm-antibody AHA is treated with glucocorticoids, other immunosuppressive agents such as rituximab, and splenectomy.
- Avoidance of cold environments may be sufficient to avoid complications of CAD. Rituximab and chemotherapy have a role, and plasmapheresis occasionally can be helpful in the acute and temporary management of symptomatic cases by physically removing the antibody.
- AHA is uncommon in children. Most cases are acute and transient, following viral infection.
- Transfusion therapy can be difficult in patients with AHA. Consultation with the blood bank is important. A history of prior pregnancy, abortion, or transfusion of blood products should be obtained, as these patients are at risk to harbor alloantibodies. No patient with AHA should succumb because serologically "compatible" RBCs are not available.

Paroxysmal nocturnal hemoglobinuria

CLINICAL CASE

A previously healthy 37-year-old female is admitted to the hospital for evaluation of severe abdominal pain. Work-up reveals mesenteric vein thrombosis. The patient is treated with thrombolytic therapy and anticoagulated with heparin, leading to clinical improvement. She has no prior or family history of thrombosis. She currently is taking an oral contraceptive. Her examination is significant for mild scleral icterus and jaundice. There is no abdominal tenderness. Mild splenomegaly is noted. Laboratory studies are significant for a hemoglobin of 106 g/L with an absolute reticulocyte count of $211 \times 10^9/L$. White count and platelet count are slightly decreased. Indirect bilirubin is elevated at 68 $\mu\text{mol}/L$, but AST, ALT, and alkaline phosphatase are normal. LDH is also increased at 1,024 U/L. Blood bank evaluation confirms a Coombs-negative hemolytic anemia. A bone marrow aspirate and biopsy showed hypercellularity and trilineage hyperplasia but no dysplasia.

PNH should be considered in the patient with unexplained hemolysis, pancytopenia, or unprovoked thrombosis. PNH is an acquired clonal disorder of hematopoietic stem cells occurring in both children and adults with no apparent familial predisposition.

Pathophysiology

PNH is a clonal disorder affecting hematopoietic stem cells arising from the somatic mutation of phosphatidylinositol glycan class A gene (*PIGA*). This results in the deficiency or absence of glycosylphosphatidylinositol (GPI) anchor on the surface of blood cells. Over 150 proteins rely on GPI to attach to the cell surface, including 2 complement regulatory proteins, CD55 (decay accelerating factor) and CD59 (membrane inhibitor of reactive lysis), which explains the unusual sensitivity of RBCs to the hemolytic action of complement. Hemolysis in PNH is due to the action of complement on abnormal RBCs. Compared with normal RBCs, PNH RBCs lyse more readily in the presence of activated complement. CD55 accelerates the destruction of C3 convertase, while CD59 inhibits the membrane attack complex. Earlier tests to diagnose PNH (eg, Ham test or acid hemolysis test; sucrose hemolysis test) were based on this property of PNH RBCs. It is now known that PNH granulocytes and platelets are sensitive to complement as well.

Whereas a *PIGA* gene mutation appears to be necessary for the development of PNH and its clinical mani-

festations, it is not sufficient because *PIGA* mutations can be found in small numbers of hematopoietic stem cells in normal individuals. Patients with aplastic anemia exhibit a larger proportion of stem cells with *PIGA* mutations. A multistep process seems necessary for PNH to develop. It is thought that in aplastic anemia, and likely in PNH, immunologic processes suppress proliferation of normal hematopoietic precursors more efficiently than proliferation of precursors lacking GPI-anchored proteins. Resistance to apoptotic death may partly explain the survival advantage of these GPI-negative cells. The abnormal clones thus are able to expand until the numbers of abnormal progeny are sufficient to cause the clinical manifestations of PNH.

Two missing GPI-linked proteins may contribute to the increased incidence of thrombosis in PNH: (1) urokinase plasminogen activator receptor, the lack of which may decrease local fibrinolysis; and (2) tissue factor pathway inhibitor, the lack of which may increase the procoagulant activity of tissue factor. PNH platelets, which are sensitive to the lytic activity of complement, are hyperactive. RBC phospholipids released during intravascular hemolysis also may initiate clotting.

Most of the clinical manifestations of the disease are due to the lack of the complement-regulating protein CD59. The monoclonal antibody eculizumab, which binds the complement component C5, thereby inhibiting terminal complement activation, decreases hemolysis of RBCs and the tendency to thrombosis as well. However, the mechanism underlying thrombosis is not yet fully elucidated. The drug does not alter the defect in hematopoiesis. Thus, although decreased hematopoiesis is probably related to deficiency of GPI-anchored proteins, it is not related to complement sensitivity.

Laboratory findings

There are no specific morphologic abnormalities of the RBCs in PNH. RBCs may be macrocytic, normocytic, or microcytic; the last occurring when iron deficiency develops because of chronic urinary iron loss from intravascular hemolysis. With or without iron deficiency, the reticulocyte count may not be as elevated as expected for the degree of anemia. This is due to underlying bone marrow dysfunction that often accompanies the PNH. Leukopenia and thrombocytopenia often are present. Serum LDH usually is elevated and may suggest the diagnosis in the patient with minimal anemia. Iron loss may amount to 20 mg/d, and urine hemosiderin often is identified. Bone marrow examination reveals erythroid hyperplasia unless there are associated bone marrow disorders.

Laboratory diagnosis

The laboratory diagnosis of PNH formerly relied on the demonstration of abnormally complement-sensitive erythrocyte populations. Ham first described the acidified serum lysis test in 1938. In that test, acidification of the serum activates the alternative pathway of the complement, and increased amounts of C3 are fixed to RBCs lacking complement regulatory proteins. Complement sensitivity of PNH RBCs also can be demonstrated in high-concentration sucrose solutions, the basis for the “sugar water” or sucrose hemolysis test. These tests are primarily of historical interest and are not used routinely in the clinical laboratory because flow cytometry techniques aimed specifically at demonstrating the deficiency in expression of GPI-anchored proteins in PNH are readily available. Using commercially available monoclonal antibodies, blood cells can be analyzed for expression of the GPI-anchored proteins CD55 and CD59. It is now also a routine to include the fluorescein-labeled aerolysin (FLAER) assay in flow cytometry, which exploits a property of aerolysin, the principal virulence factor of the bacterium *Aeromonas hydrophila*. FLAER binds selectively with high affinity to the GPI anchor of most cell lineages. These flow cytometric methods have the sensitivity to detect small abnormal populations; because monocytes and granulocytes have short half-lives and their numbers are not affected by transfusion, analysis of GPI-anchored proteins on neutrophils or monocytes rather than RBCs is preferred.

Clinical manifestations

The clinical manifestations of PNH are highly variable among patients. Although chronic hemolytic anemia is a common manifestation, only a minority of patients report nocturnal hemoglobinuria. The degree of anemia seen in PNH varies in affected individuals from minimal to quite severe. The anemia can be due to hemolysis, iron deficiency from urinary iron loss, or an associated bone marrow failure condition. Symptoms related to episodes of hemolysis include back and abdominal pain, headache, and fever. Exacerbations of hemolysis can occur with infections, surgery, or transfusions. Several symptoms in PNH may be related to the ability of free plasma hemoglobin to scavenge NO, as previously discussed.

Aplastic anemia has been diagnosed both before and after the identification of PNH. PNH clones are present in approximately 20% of patients with severe aplastic anemia. Approximately 20% of patients with myelodysplastic syndromes have PNH clones. Hemolysis in the setting of bone marrow hypoplasia should suggest the diagnosis of PNH. Infections associated with leukopenia and bleeding

due to thrombocytopenia contribute to increased mortality. An increased incidence of acute leukemia also has been reported. While PNH clones are found commonly in myelodysplastic syndrome, they are generally transient and not clinically relevant.

Patients frequently have thrombotic complications that can be life-threatening and may represent the initial manifestation of PNH. In addition to venous thrombosis involving an extremity, there is a propensity for thrombosis of unusual sites such as hepatic veins (Budd-Chiari syndrome), other intra-abdominal veins, cerebral veins, and venous sinuses. Thus, complaints of abdominal pain or severe headache should alert the clinician to the consideration of thrombosis in the patient with PNH. The thrombotic tendency is particularly enhanced during pregnancy.

Treatment

For patients who have mild hemolysis and are asymptomatic (usually clone size <10%), no clinical intervention is needed. Folate supplementation is generally recommended regardless of whether a treatment is indicated or not. Because expansion of the clone may occur, the size of the clone may be monitored every 12 months. Currently, the most effective treatments are allogeneic hematopoietic stem cell transplantation and eculizumab.

Allogeneic hematopoietic stem cell transplantation is the only cure for PNH. However, because of the high risk for serious complications including death, it should not be offered as the initial treatment. Rather, it should be reserved for patients with no access to eculizumab, severe aplastic anemia, or the rare individuals whose hemolysis or thrombosis is not controlled by eculizumab. For patients with PNH and marrow failure who lack an HLA-matched sibling donor, immunosuppressive therapy may be attempted.

Eculizumab is approved in PNH to treat hemolysis based on efficacy in 2 phase-3 clinical trials. Eculizumab reduces intravascular but not extravascular hemolysis, eliminates or reduces transfusion requirement in most patients, improves quality of life, ameliorates pulmonary hypertension, and decreases the risk of thrombosis. However, it does not treat the marrow failure or the underlying cause of PNH and must be used indefinitely.

Although eculizumab is generally well tolerated, its most serious complication is sepsis due to *Neisseria* organisms. Patients congenitally lacking one of the terminal complement components, C5 to C9, are known to be at risk for *Neisseria* infection. Patients receiving eculizumab are at risk because of its inhibition of the terminal complement sequence. Vaccination against *Neisseria meningitidis* is

recommended 2 weeks before starting therapy. Revaccination every 3 to 5 years may be important because eculizumab is given for an indefinite period. Because vaccination does not eliminate the risk completely, patients should be told to seek medical attention for any symptoms consistent with *Neisseria* infection. Antibiotic prophylaxis is necessary if eculizumab has to be administered less than 2 weeks after vaccination.

Thrombosis is the leading cause of death in PNH patients. It should be treated promptly with anticoagulation. Thrombolytic therapy may be considered as well, depending on the extent and location of the clot. In contrast to anticoagulation as treatment, prophylactic anticoagulation is controversial. In one large, nonrandomized trial, primary prophylaxis with warfarin decreased the risk of thrombosis in patients with large PNH clones (>50% PNH granulocytes). Because eculizumab also decreases the risk of thrombosis, prophylactic anticoagulation is not indicated in these patients. The question remains as to whether prophylactic anticoagulation is beneficial in patients who do not require eculizumab. The exception may be pregnant women who are at particularly increased risk for thrombosis; low-molecular-weight heparin may be useful in these patients during pregnancy and the puerperal period. Eculizumab crosses the placenta and is present in cord blood. However, its use during pregnancy is apparently safe and appears to reduce fetal mortality and maternal morbidity. Also, patients with PNH undergoing surgery should receive prophylactic anticoagulation in the perioperative period.

Prognosis

The median survival for PNH is 10 to 15 years. Thrombotic events, progression to pancytopenia, and age >55 years at diagnosis are poor prognostic factors. The development of a myelodysplastic syndrome or acute leukemia markedly shortens survival. Patients without leukopenia, thrombocytopenia, or other complications can anticipate long-term survival.

is treated with anticoagulation; thrombolytic therapy may be employed if the thrombosis is acute. There are no randomized studies to support anticoagulation for prophylaxis of thrombosis, but it is prudent to employ prophylaxis in high-risk situations for thrombosis, such as pregnancy or surgery. If pancytopenia is marked, immunosuppressive therapies, such as antithymocyte globulin and cyclosporine, have been used. Allogeneic marrow transplantation has been performed in selected cases, primarily those with severe marrow failure and an HLA-matched sibling donor. Marrow transplantation is the only potentially curative therapy of PNH.

KEY POINTS

- PNH is an acquired clonal hematopoietic stem cell disorder caused by a somatic mutation of the *PIGA* gene that results in hematopoietic cells lacking GPI-linked proteins.
- Patients may experience chronic hemolytic anemia, cytopenias, or a thrombotic tendency.
- Flow cytometric techniques to identify cell populations lacking GPI (FLAER) or GPI-linked proteins (CD55 and CD59) are the standard diagnostic tests.
- PNH clones have been identified in individuals without hematologic abnormalities.
- Bone marrow failure often precedes or follows clinical PNH.
- Eculizumab, a monoclonal antibody directed against C5, eliminates or reduces hemolysis, improves quality of life, and decreases the risk of thrombosis.
- *Neisseria* sepsis is a potentially fatal complication of eculizumab therapy. Vaccination against *Neisseria* should be given 2 weeks before initiation of eculizumab. Antibiotic prophylaxis is necessary if eculizumab has to be administered less than 2 weeks after vaccination.
- Prompt evaluation is indicated for symptoms of thrombosis, particularly at unusual sites. Anticoagulation is indicated for documented thrombosis and thrombolytic therapy may be useful, depending on the location and size of the clot.
- Prophylactic warfarin seems to prevent thrombosis in patients with large PNH clones, but its use for this purpose is controversial, at least in patients who respond to eculizumab.
- Allogeneic hematopoietic cell transplantation has curative potential. Because of the risk of serious or fatal complications, its use should be reserved for those patients with severe cytopenias or patients with severe hemolysis or thrombosis refractory to eculizumab.

CLINICAL CASE (continued)

The patient presented in this section likely has PNH. She has evidence of hemolysis and marrow failure. The diagnosis can be confirmed by flow analysis for FLAER, CD55, and CD59 on granulocytes, revealing a population of cells with absence of GPI or GPI-linked proteins. Treatment is aimed at the major clinical presentation. Eculizumab is effective in decreasing hemolysis and thrombosis, but not marrow failure. Thrombosis

Fragmentation hemolysis

CLINICAL CASE

A 63-year-old male is referred for evaluation of anemia. His past history is significant for oxygen-dependent chronic obstructive pulmonary disease, coronary artery disease, a mechanical aortic valve placed in 1986, and mild heart failure. On examination, he has distant breath sounds and a grade III/VI systolic ejection murmur heard at the left upper-sternal border. Mild scleral icterus is noted. Laboratory data are significant for a hemoglobin of 70 g/L (normal 2 years prior). Absolute reticulocyte count is elevated at $176 \times 10^9/\text{L}$, LDH 1,686 IU/dL, and indirect bilirubin 58 $\mu\text{mol}/\text{L}$. Examination of the blood smear reveals schistocytes, hypochromic RBCs, and a few cigar-shaped RBCs.

Fragmentation hemolysis takes place within the vasculature. Laboratory features common to both intra- and extravascular hemolysis include increased concentrations of plasma bilirubin and LDH and decreased concentration of plasma haptoglobin. Additional features characteristic of intravascular as opposed to extravascular hemolysis include the presence of free hemoglobin in the plasma and urine, resulting in red urine and pink plasma. If the hemolysis is chronic, urine hemosiderin may be present. In fragmentation hemolysis, schistocytes are a prominent feature of the blood smear (Figure 8-9). The differential diagnosis of fragmentation hemolysis is summarized in Table 8-8.

Figure 8-9 Schistocytes. Source: ASH Image Bank/Peter Maslak (image 00003718).

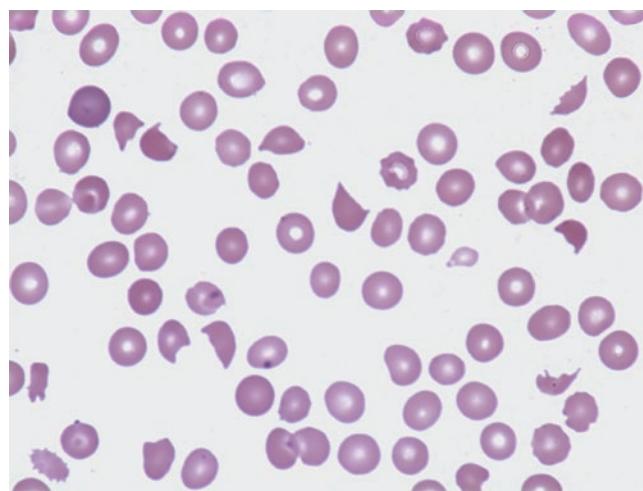


Table 8-8 Differential diagnosis of fragmentation hemolysis

Atypical hemolytic uremic syndrome
Cardiac valve disease
Disseminated intravascular coagulation
HELLP syndrome
Hemolytic uremic syndrome
Malignancy
Malignant hypertension
Scleroderma renal crisis
Thrombotic thrombocytopenia purpura
Vasculitis

Pathophysiology

Among the several causes of fragmentation hemolysis, the common thread is mechanical damage to RBCs, resulting in the presence of fragmented RBCs or schistocytes on the blood smear. When microvascular or endothelial injury is present, the process is termed microangiopathic hemolytic anemia (MAHA). When thrombosis is part of the picture, the term thrombotic microangiopathy is used. In disseminated intravascular coagulation (DIC), the MAHA is accompanied by activation and consumption of soluble clotting factors, resulting in prolongation of the prothrombin time and activated partial thromboplastin time; whereas TTP and hemolytic uremic syndromes (HUS) are associated with activation of platelets but not soluble clotting factors.

Injury to blood vessel endothelium, intravascular clotting, and primary platelet activation all result in formation of fibrin strands in the circulation. The shearing force generated as the RBCs pass through the fibrin strands causes the RBCs to be cut into small irregular pieces. RBCs may be broken into pieces by direct mechanical trauma as may occur in march hemoglobinuria or with a dysfunctional mechanical heart valve in which high-velocity jets of blood strike a non-endothelialized surface. The resulting small RBC fragments are self-sealing and continue to circulate, albeit with shortened survival. This is due in part to their decreased deformability, which results in accelerated removal by the spleen.

Etiology

Cardiac valve hemolysis

Hemolysis may occur with calcific or stenotic native heart valves, although it is usually very mild and well compensated in the absence of severe valvular disease. Mechanical heart valves have a smaller diameter than the native heart valve. Normally, the hemodynamic consequences are minimal. However, prosthetic valve dysfunction or perivalvular regurgitation may result in intravascular hemolysis. An aged

or damaged valve surface may become irregular, leading to thrombus formation. In a high-flow state, such as exists across the aortic valve or across a regurgitant mitral valve, the formation of jets and turbulent flow results in high shear stress that may exceed the stress resistance of the normal RBC. Hemolysis may be made worse with concomitant cardiac failure or high-output states. Recently designed bioprosthetic heart valves have a significantly decreased risk of thrombus formation and a lower rate of traumatic hemolysis. A recent prospective study reported a 25% rate of mild subclinical hemolysis with a mechanical prosthesis and a 5% rate with a bioprosthetic.

Ruptured chordae tendinae, aortic aneurysm, and patch repair of cardiac defects, as well as intraventricular assist devices and aortic balloon pumps used in the management of severe heart failure, have been associated with traumatic hemolysis. Intravascular hemolysis has been described after cardiopulmonary bypass and is thought to be secondary to both physical damage and complement activation. Anemia is variable in patients with prosthetic valve hemolysis. The blood smear usually shows schistocytes. However, the schistocytosis may not be prominent.

With chronic hemolysis, hemoglobin is lost in the urine, leading to iron deficiency. Iron-deficient RBCs are mechanically fragile, which can worsen hemolysis, exacerbate anemia, and lead to further hemodynamic compromise that may increase the rate of hemolysis. At times, this cycle may be abated by correction of iron deficiency or by RBC transfusion. The addition of erythropoietin to increase RBC production may compensate for ongoing hemolysis. If anemia is severe or fails to respond to the conservative measures, valve replacement may become necessary.

Thrombotic thrombocytopenic purpura

TTP is due to the deposition of platelet microthrombi along the endothelium of small vessels of multiple organs. The classic clinical presentation consists of MAHA and thrombocytopenia. In advanced stages, fever, renal failure, and CNS involvement are seen. TTP may be confused with eclampsia, HUS, atypical HUS (aHUS), the HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, and acute fatty liver of pregnancy (AFLP), all of which can present with microangiopathic anemia. A critical distinguishing feature between TTP and DIC is the presence of consumptive coagulopathy in the latter. Malignant hypertension and renal crisis in scleroderma may resemble TTP, presenting with microangiopathic hemolysis, thrombocytopenia, and renal insufficiency. Rapid control of hypertension is important in these patients (TTP is covered in detail in Chapter 11).

Certain drugs, especially antineoplastic agents, can cause microangiopathic hemolysis that resembles TTP. Mitomycin, a chemotherapeutic agent used in the treatment of gastrointestinal malignancies, has been best described. Gemcitabine, another chemotherapeutic agent, also has been implicated. The mechanism has been proposed to be direct endothelial injury. Both tacrolimus and cyclosporine used to prevent and treat graft-versus-host disease can cause a similar syndrome. Both ticlopidine and clopidogrel, antiplatelet agents, have been associated paradoxically with TTP.

Hemolytic uremic syndromes

HUS is characterized by red cell fragmentation, thrombocytopenia, and renal failure. HUS has been divided into typical and atypical forms. The typical form is usually caused by infection with *Escherichia coli*. aHUS is due to dysregulation of the complement alternative pathway and is becoming increasingly recognized with the ability to distinguish this cause of MAHA and thrombocytopenia from TTP with the use of ADAMTS13 testing. Individuals with aHUS do not always have thrombocytopenia, as seen in a French registry of patients where 16% did not have a low platelet count at presentation. It is now thought that aHUS occurs in individuals with an underlying complement risk factor who have a secondary trigger. Triggers can include drug exposure, infection, malignancy, pregnancy, and surgery.

Distinguishing TTP or other MAHA from aHUS is crucially important, as therapy with eculizumab has been shown to be more effective than plasma exchange in the management of aHUS. Now approved for aHUS, eculizumab has been shown to result in full recovery of baseline renal function in 80% of children and 31% of adults. In a patient with MAHA and thrombocytopenia with normal ADAMTS13 levels, eculizumab therapy should be considered (aHUS is covered in detail in Chapter 11).

Disseminated intravascular coagulation

DIC is associated with many disorders, including sepsis, obstetrical catastrophes, and malignancy. The disorder is characterized by activation of coagulation and generation of excess thrombin leading to deposition of fibrin strands in arterioles, venules, and capillaries. MAHA may be present, but often is not severe enough to cause morbidity. Disseminated malignancy presents with MAHA and DIC in approximately 5% of cases. Fibrin deposition and vascular disruption by the malignancy itself have both been noted. Mucin-producing adenocarcinomas are frequent offenders. Promyelocytic leukemia characteristically presents with DIC due, at least in part, to the release of tissue

factor from promyelocytic granules. If treatment is effective at reversing the underlying condition causing DIC, hemolysis and the coagulopathy often resolve.

HELLP syndrome

The HELLP syndrome, which is a serious complication of late pregnancy, is part of a spectrum including preeclampsia. Thrombocytopenia and MAHA with or without renal failure may also occur in pregnancy due to TTP, aHUS, and AFLP. It is important to distinguish TTP, aHUS, and AFLP from HELLP and preeclampsia for therapeutic reasons. The clinical features are quite similar, however, and the correct diagnosis is often elusive.

Although not absolute, the timing of onset during the pregnancy may be helpful. In general, TTP and aHUS occur earlier in gestation than AFLP, preeclampsia, or HELLP. Approximately two-thirds of TTP cases in pregnancy occur in the first or second trimester. Most cases of AFLP, preeclampsia, and HELLP occur after 20 weeks of gestation, the great majority in the third trimester. A history of proteinuria and hypertension before the onset of hemolysis, liver abnormalities, and thrombocytopenia favors the diagnosis of preeclampsia or HELLP, whereas a high LDH level with only modest elevation of AST favors TTP. Severe liver dysfunction or liver failure favor AFLP.

The characteristics of the coagulopathy are different as well. Whereas both TTP and HELLP are characterized by thrombocytopenia, in HELLP and more so in AFLP, DIC may also be present with evidence of consumptive coagulopathy. In TTP, only thrombocytopenia is seen without evidence of consumption of soluble clotting factors. Treatment of HELLP and AFLP consists of prompt delivery of the fetus. The use of dexamethasone in HELLP, previously supported by small studies, has not proven helpful in subsequent randomized trials.

Kasabach-Merritt syndrome

Kasabach-Merritt syndrome is characterized by consumptive coagulopathy occurring in the capillaries of a large kaposiform hemangioendothelioma. MAHA accompanies evidence of DIC. A number of treatments, including glucocorticoids, chemotherapy, interferon-alfa, embolization, and surgical removal have been tried with some success.

Foot strike hemolysis

Foot strike hemolysis, also known as march hemoglobinuria, has been described in soldiers subjected to long foot-stomping marches in rigid-soled boots, long-distance runners, conga drummers, pneumatic hammer operators, and karate enthusiasts. Hemoglobinuria occurs shortly

after the episode of exercise. The hemolysis is caused by direct trauma to RBCs in the blood vessels of the extremities. This condition has become much less common as shoe technology has improved. Cessation of the activity always leads to resolution of the hemolysis.

CLINICAL CASE (continued)

The patient presented in this section has evidence of a moderate hemolytic anemia. The blood smear is consistent with both traumatic hemolysis and iron deficiency, as schistocytes and hypochromic and cigar-shaped cells were noted on review of the peripheral blood smear. Valve structure and function should be investigated with an echocardiogram or other imaging studies. Other causes for hemolysis should be ruled out. The patient should be evaluated for iron deficiency. If further evaluation confirms iron deficiency, the patient should receive iron replacement therapy. Erythropoietin administration may also be considered once iron store is replete. He appears to be a poor surgical candidate, but valve replacement may become necessary if conservative treatment fails.

Hemolytic anemia due to chemical or physical agents

CLINICAL CASE

A 23-year-old female is referred for evaluation of mild anemia noted during a work-up of liver function test abnormalities. Her recent history has been significant for bizarre schizophrenia-like behavior and arthritis. She has not had a menstrual period in several months. Recent slit-lamp examination by an ophthalmologist revealed golden brown pigmentation of the cornea. Physical examination is otherwise unremarkable. Laboratory data suggest a DAT-negative hemolytic anemia. Liver enzymes are moderately elevated. A ceruloplasmin level returns low at 110 µmol/L.

The use of primaquine and dapsone to prevent or treat *Pneumocystis jirovecii* in patients with AIDS has become fairly common. Both drugs may cause methemoglobinemia in high doses in normal patients and may precipitate hemolysis in patients with G6PD deficiency. Most AIDS clinics screen their patients for G6PD deficiency before starting either of these drugs.

Ribavirin, used to treat HCV infection, is a frequent cause of hemolysis by an unknown mechanism. The hemolysis is dose dependent and decreases or resolves with decreased ribavirin dose or discontinuation of the drug. The rate of sustained HCV response, however, also decreases

with dose reduction. Erythropoietin has been used as an adjunct to maintain ribavirin therapy at full dose.

Phenazopyridine is a bladder analgesic that is used to treat the symptoms of cystitis. In high doses, it has been associated with oxidative hemolysis. It is recommended that patients be treated for no more than 2 days. Overdoses, prolonged administration, and renal insufficiency have led to methemoglobinemia and severe hemolysis, occasionally severe enough to induce acute renal failure.

Lead intoxication can lead to a modest shortening of RBC life span, although the anemia more often is due to an abnormal heme synthesis and decreased production of erythrocytes. On the blood smear, RBCs are normocytic and hypochromic, with prominent basophilic stippling in young polychromatophilic cells.

Copper causes hemolysis through direct toxic effects on RBCs and has been observed in association with hemodialysis. Copper accumulates in RBCs and disrupts normal metabolic function through a variety of mechanisms, including oxidation of intracellular reduced glutathione, hemoglobin, and NADPH and inhibition of multiple cytoplasmic enzymes. Wilson disease, due to a mutation of the ATP7B gene, leads to absence or dysfunction of a copper-transporting ATPase encoded on chromosome 13. This subsequently results in lifelong copper accumulation. Hemolytic anemia may be an early manifestation. The hemolytic process in Wilson disease varies in severity and duration. Kayser-Fleischer rings due to the deposition of copper around the periphery of the cornea are a key diagnostic finding. Diagnosis can be made by quantitative ceruloplasmin measurements or by liver biopsy with assessment of the copper concentration. Treatment consists of penicillamine, which mobilizes copper stores. Acute hemolysis in Wilson disease has been treated successfully with plasmapheresis.

Certain spider bites may be associated with traumatic RBC fragmentation. In the southern United States, the brown recluse spider (*Loxosceles reclusa*) is the most common species causing hemolysis. The toxin proteolyzes the RBC membrane through damage to protein band 3 and other integral proteins. In the northwestern United States, hemolysis has been noted after hobo spider (*Tegenaria agrestis*) bites. Microangiopathic hemolysis may occur after the bite of pit vipers (eg, rattlesnakes, cottonmouth moccasins, and copperheads) associated with DIC induced by the venom. Cobra venom contains phospholipases that may cause hemolysis. Massive bee and wasp stings rarely have been associated with intravascular hemolysis.

Fragmentation hemolysis has been described after injury from a variety of physical agents. Thermal injury can lead to severe intravascular hemolysis. This is best described in patients suffering from extensive third-degree burns. At

temperatures above 47°C, irreversible injury occurs to the RBC membrane. Shortened RBC survival has been noted after ionizing radiation exposure.

CLINICAL CASE (continued)

The patient presented in this section displays the classic historical and physical findings of Wilson disease. The low ceruloplasmin level is diagnostic. Hemolytic anemia has been well described in this disease. Once the severity of her liver disease is further evaluated, treatment with penicillamine should be considered. The hemolytic anemia is likely to resolve as excess copper is removed.

Hemolytic anemia due to infection

CLINICAL CASE

A 21-year-old man went to the emergency department of his local hospital complaining of fever and shaking chills. He had just returned from a 6-month deployment in eastern Afghanistan with the US Army. He has been home for 2 weeks on leave before reporting for his next duty assignment in the United States. He states that he faithfully took his malaria prophylaxis consisting of mefloquine 250 mg weekly while in Afghanistan. He was instructed to continue the weekly mefloquine for 4 more doses postdeployment, plus primaquine 15 mg daily for the first 2 weeks. On examination, he appeared acutely ill. His vital signs were BP 126/66, pulse 110, respiration 20, and temperature 39°C. The remainder of the examination was unremarkable. There was no splenomegaly. A Wright-Giemsa stained thick blood smear confirmed the diagnosis of *Plasmodium vivax* malaria.

Infection may lead to hemolysis through a variety of mechanisms. Parasites may directly invade RBCs, leading to premature removal by macrophages of the liver and spleen. Alternatively, hemolytic toxins may be produced by the organism and lead to damage of the RBC membrane. Development of antibodies to RBC surface antigens has been well described with certain viral and bacterial illness, especially infectious mononucleosis and *Mycoplasma pneumoniae* infections. Hypersplenism may ensue, which can further decrease RBC life expectancy. In addition, the antibiotic drugs used to treat a variety of these infections may lead to further hemolysis in G6PD-deficient individuals. Anemia that occurs with concomitant acute or chronic infection is likely to be multifactorial, with the anemia of chronic inflammation often coexisting and predominating.

RBC membrane injury caused by bacteria

Clostridial sepsis

Sepsis from *Clostridium perfringens* or *Clostridium septicum* is seen in patients with anaerobic subcutaneous infections, in body areas of impaired circulation, after trauma, after septic abortion or postpartum sepsis, and in patients with acute cholecystitis with gangrene of the gallbladder or bowel necrosis. It has a mortality rate of almost 75%. Severe neutropenia is a risk factor. The α toxin of *Clostridium* is a lecithinase (phospholipase C) that disrupts the lipid bilayer structure of the RBC membrane, leading to membrane loss and massive hemolysis. A common clinical scenario is the inability of the phlebotomist in the emergency room to draw a nonhemolyzed blood sample despite multiple attempts. The plasma may be a brilliant red color, and there may be dissociation between the hemoglobin and hematocrit values because of the plasma hemoglobin levels reaching several grams per deciliter. Acute renal failure, secondary to excessive hemoglobinuria, may ensue. Renal failure and hepatic failure contribute to the high mortality in clostridial sepsis.

Hemolytic anemias with gram-positive and gram-negative organisms

Septicemia and endocarditis caused by gram-positive bacteria, such as streptococci, staphylococci, *Streptococcus pneumoniae*, and *Enterococcus faecalis* are often associated with hemolytic anemia. The anemia in patients with infections due to gram-positive cocci appears to result from the direct toxic effect of a bacterial product on erythrocytes. *Salmonella typhi* infection may be accompanied by severe hemolytic anemia with hemoglobinemia. In typhoid fever, the onset of hemolysis may occur during the first 3 weeks of illness, with anemia lasting from several days to 1 week. *Salmonella* and other microorganisms can cause direct agglutination of RBCs in vitro, but it is not known whether this phenomenon contributes to *in vivo* hemolysis. In approximately one-third of patients with typhoid fever, a positive DAT develops, but hemolytic anemia is not manifest in all cases.

Immune hemolysis associated with infections

Pneumonia caused by *M. pneumoniae* can be associated with production of cold agglutinins, IgM antibodies directed against the RBC I antigen. Hemolytic anemia associated with *Mycoplasma* may occur during the second or third week of the illness. The onset of the hemolysis may be rapid, usually occurring after recovery from respiratory symptoms. The clinical presentation often includes dyspnea or fatigue and the presence of pallor and jaundice. The blood smear shows RBC agglutination with or without spherocytosis and with polychromatophilia (Figure 8-7). When EDTA-anticoagulated blood is cooled in a test tube, RBC

agglutination can be seen; disagglutination occurs when the blood is warmed. Cold agglutinin titers at the onset of hemolysis usually exceed 1:256 and may reach higher levels, although they are typically lower than in monoclonal cold agglutinin disease. The DAT is positive for complement deposition on RBCs. The hemolytic anemia associated with *Mycoplasma* pneumonia is self-limited, transient, and usually mild, although severe cases requiring corticosteroid therapy or plasmapheresis have been reported.

Infectious mononucleosis caused by Epstein-Barr virus infection may be associated with hemolytic anemia due to cold agglutination. The cold agglutinin in this case is an IgM antibody directed against the i antigen. Severe hemolytic anemia associated with infectious mononucleosis is unusual, although anti-i antibodies frequently are present. When hemolytic anemia occurs, the mechanism involves fixation of complement on the RBC membrane by IgM antibodies. Hemolysis proceeds either by completion of the complement cascade through C9 or by opsonization of RBCs with fragments of C3 leading to phagocytosis of RBCs by macrophages in the liver or spleen.

Several other viral infections have been associated with AHA. These include cytomegalovirus, herpes simplex, rubella, varicella, influenza A, and HIV. Postviral acute hemolytic anemia in children may be due to PCH, in which a cold-reactive hemolytic IgG antibody of the Donath-Landsteiner type induces complement lysis of RBCs. Patients with either congenital or tertiary syphilis may also develop paroxysmal cold hemoglobinuria. Whereas PCH used to be fairly common in the late 19th and earlier 20th centuries, it is rare in the 21st century due to the disappearance of congenital and tertiary syphilis.

MAHAs associated with infection include bacteremia with gram-negative organisms, staphylococci, meningococci, and pneumococci, all of which can lead to DIC with endothelial damage and fibrin thrombi within the microcirculation. RBC injury results from mechanical fragmentation by fibrin strands in the vasculature. Microvascular damage induced by meningococcal and rickettsial infections (eg, Rocky Mountain spotted fever) may be associated with DIC, thrombocytopenia, and microvascular thrombi.

Hemolytic anemia associated with parasitic infestation of RBCs

Malaria

Malaria is the most common cause of hemolytic anemia worldwide. Transmitted by the bite of an infected female *Anopheles* mosquito, sporozoites that are injected from the mosquito make their way to liver cells. Merozoites enter into the bloodstream 1 to 2 weeks later. Hemolysis in malaria results directly from RBC infestation by *Plasmodium* organisms (Figures 8-10 and 8-11). Noninfected RBCs

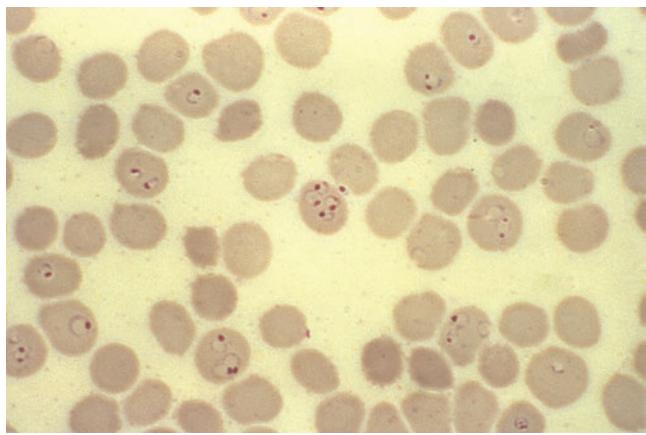


Figure 8-10 The intraerythrocyte parasite *Plasmodium falciparum*. Source: Centers for Disease Control and Prevention (CDC) Public Health Image Library (phil.cdc.gov)/Steven Glenn.

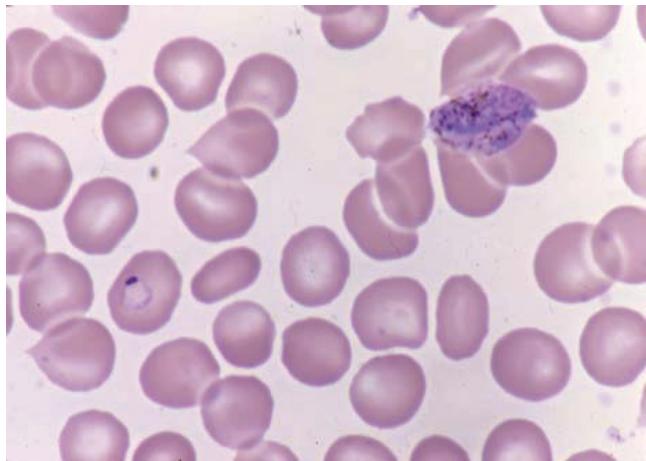


Figure 8-11 The intraerythrocyte parasite *Plasmodium vivax*: trophozoite (ring form) and female gametocyte.

may be hemolyzed by poorly understood mechanisms. Infested RBCs are selectively removed from the circulation by the spleen, with some RBCs reentering circulation after splenic pitting of parasites. Previously infested RBCs manifest membrane and metabolic abnormalities along with decreased deformability. In addition, the *Plasmodium* species digests the host RBC hemoglobin for its own use as a nutrient.

The severity of the hemolytic process is often out of proportion to the degree of parasitemia. *P. vivax* and *Plasmodium ovale* invade only reticulocytes, whereas *Plasmodium malariae* invades only mature erythrocytes. *P. falciparum* invades erythrocytes of all ages and is associated with more severe hemolysis. In areas where malaria has been a frequent cause of death for many centuries, a number of genetic polymorphisms are prevalent, including G6PD deficiencies, thalassemias, and hemoglobinopathies. These polymor-

phisms have in common the ability to interfere with the ability of the malaria parasites to invade RBCs.

With *P. falciparum* infection, intravascular hemolysis may be severe and associated with hemoglobinuria (blackwater fever). Another potentially lethal complication of *P. falciparum* infection, cerebral malaria, results from expression of a combination of parasite-induced RBC surface proteins including *P. falciparum* erythrocyte protein 1. These RBCs adhere to receptors on vascular endothelium in various organs, including the central nervous system, resulting in vaso-occlusion and neurologic manifestations.

Diagnosis of malaria is based on identification of parasite-infected RBCs on a thick Wright-stained blood smear. The distinction of *P. falciparum* infection from the other species is important because its treatment may constitute a medical emergency. The findings of 2 or more parasites per RBC and infestation of >5% of RBCs are characteristic of *P. falciparum* infection.

Chemoprophylaxis should be offered to all people planning travel to known endemic areas. The hemolytic anemia of malaria resolves after successful therapy with quinine, chloroquine, artemisinin, and other drugs, depending on the species of malaria. Many of these agents may be associated with drug-induced hemolysis in patients with G6PD deficiency.

Babesiosis

Babesiosis is a protozoan infection caused by *Babesia microti*. Once thought to be rare, outbreaks have been described with increasing frequency on Nantucket Island and in Cape Cod, northern California, and several other North American locations. The organism is transmitted by the bite of the *Ixodes* tick, which infects many species of wild birds and domestic animals and occasionally humans. Babesiosis rarely may be transmitted by transfusion with fresh or frozen-thawed RBCs. Infection leads to a clinical syndrome of fever, lethargy, malaise, and hemoglobinuria 1 to 4 weeks after the bite. Hemolytic anemia due to intravascular hemolysis occurs, and renal and liver function tests are frequently abnormal. The disease is often asymptomatic in people with intact spleens; patients who have undergone splenectomy are at high risk for severe symptomatic infection. *Babesia* infection can be diagnosed by demonstrating typical intraerythrocytic parasites on a thin blood smear (Figure 8-12). The standard treatment is atovaquone plus azithromycin. A warm AHA can manifest 2 to 4 weeks after diagnosis and may require immunosuppressive treatment.

Bartonellosis

Bartonellosis, caused by *Bartonella bacilliformis*, manifests in 2 clinical stages: an acute hemolytic anemia and a chronic granulomatous phase. The microorganism enters

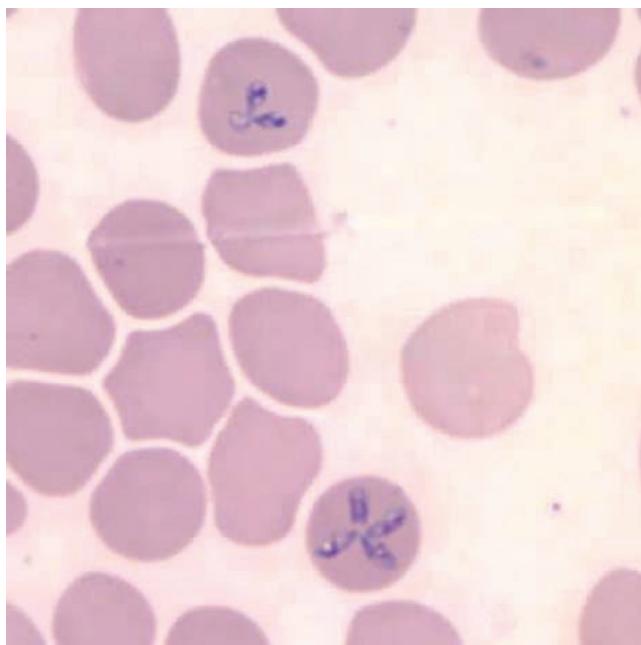


Figure 8-12 The intraerythrocyte parasite *Babesia microti* (Maltese cross formation).

the blood following the bite of an infected sand fly. The infective *Bartonella* agent adheres to the membrane of RBCs that are then removed by the spleen. The hemolytic anemia of bartonellosis develops rapidly and may be severe, with hemoglobinemia and hemoglobinuria. When untreated, this disorder is associated with high mortality. Survivors manifest a second stage of the disease with cutaneous granulomas. Bartonellosis is common in South America and has been reported in the Peruvian Andes and parts of Brazil, where it is also known as Oroya fever. On Giemsa-stained blood films, red-violet rods of varying lengths can be identified on RBCs and represent the bacteria. Effective treatment exists and consists of penicillin, streptomycin, chloramphenicol, or tetracycline.

CLINICAL CASE (continued)

The patient was admitted for treatment. The CDC Malaria Hotline (1-770-488-7788) was called, and the regimen of chloroquine and primaquine was recommended for vivax malaria acquired in Afghanistan. He made a full recovery. He ultimately admitted that he had forgotten to take his prophylactic medications after leaving Afghanistan. The most common cause of failure of malaria prophylaxis in military or civilian populations is noncompliance. Because of the importance of primaquine in terminal prophylaxis and treatment of vivax malaria, it is currently the policy of the US military to screen all personnel for G6PD deficiency.

Hemolysis from other causes

Several other conditions have been described to be associated with hemolytic anemia. These are generally of rare occurrence. Vitamin B₁₂ deficiency rarely (1%) presents as hemolytic anemia, but usually with concomitant thrombocytopenia or neutropenia. Ineffective marrow erythropoiesis and pronounced hyperhomocysteinemia have been postulated to be potential causes of hemolysis. In patients with liver failure, biochemical changes may affect the integrity of the red cell membrane either structurally or metabolically, leading to premature RBC destruction. Iatrogenic causes such as administration of intravenous immunoglobulin and anti-D may lead to hemolysis, but they are self-limited and frequently mild. Congenital dyserythropoietic anemia types II and III also present with hemolytic anemia. They are extremely rare bone marrow failure syndromes characterized by failure of terminal erythropoiesis. Congenital dyserythropoietic anemia is covered in Chapter 16.

KEY POINTS

- RBC fragmentation syndromes are diverse in etiology.
- In all suspected cases of hemolytic anemia, the blood smear should be examined carefully for schistocytes. Their presence can direct differential diagnosis.
- RBC destruction can be at the macrovascular or microvascular (microangiopathic) level of the circulatory system. Classic examples include heart valve hemolysis, DIC, and TTP.
- Various chemical exposures or physical agents can cause fragmentation hemolysis.
- Infection can cause accelerated RBC destruction through a variety of mechanisms, including direct invasion, toxin production, and immune mechanisms.
- Malaria, the most common infectious disease worldwide, causes hemolysis through both direct parasitic invasion of RBCs and alterations in noninfected cells. It can be diagnosed by thorough review of a thick Wright-stained peripheral blood smear.

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Thrombosis and thrombophilia

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Introduction

This chapter reviews the epidemiology as well as various clinical, diagnostic, and therapeutic aspects of thrombosis; discusses the drugs used as antithrombotics; pathophysiologic contributors to thrombosis; and describes the mechanisms, testing issues, and clinical relevance of inherited and acquired thrombophilias.

Venous thromboembolism

With an incidence of 2 to 3 per 1,000 per-person year, estimates suggest that between 300,000 to 600,000 people in the United States develop deep vein thrombosis/pulmonary embolism (DVT/PE) each year, and that at least 60,000 to 100,000 deaths each year are due to venous thromboembolism (VTE). The incidence increases with age, up to 2 to 7 per 1,000 in those over the age of 70. Approximately half of DVT/PE episodes are hospital associated, with VTE being the leading (in low- and middle-income countries) or second most common (in high-income countries) cause of disability-adjusted life-years lost.

In children, the incidence of VTE is 0.07 to 0.14 per 10,000. However, if one considers hospitalized children, the rate increases by 100 to 1,000 times to at least 58 per 10,000 admissions. Therefore, despite some exceptions, venous thrombosis should be considered a disease of sick children. The commonest age groups for VTE are neonates and teenagers, and this reflects the pattern of associated underlying diseases and interventions. The most common precipitating factor is the presence of central venous access devices (CVADs), which are related to almost 90% of VTE in neonates and more than 60% in older children. CVADs are common in the care of children with cancer, cardiac defects requiring surgery, pediatric and neonatal intensive care, and those requiring parenteral nutritional support. Thus, a large proportion of VTE in children occurs in the upper venous system (subclavian veins, internal jugular veins, brachiocephalic veins) in accordance with placement of a CVAD.

Deep vein thrombosis of the leg and pulmonary embolism

Symptoms

The term *DVT* refers to thrombosis involving deep veins of either the leg (popliteal, femoral, iliac) or arm (brachial, axillary, subclavian, and brachiocephalic).

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DVT of the pelvic and leg veins presents with varying degrees of leg swelling, pain, warmth, and skin discoloration. Symptoms are typically nonlocalized in the leg, and localized symptoms are more suggestive of a superficial thrombophlebitis. It is important to recognize that *proximal* DVT is defined as involving the popliteal or more proximal (eg, femoral or iliac) veins, whereas *distal* DVT involves vessels distal to the trifurcation of the popliteal vein. A palpable subcutaneous cord-like firmness is indicative of a superficial thrombophlebitis and is discussed in a separate paragraph. The onset of symptoms of DVT can be sudden or subacute over days to weeks. DVT can easily be missed or misdiagnosed, as the symptoms can be nonspecific. PE presents with varying degrees of severity of shortness of breath, chest pain that is classically respiratory dependent, nonproductive cough, and hemoptysis. A massive PE can lead to sudden death. Small PEs are often asymptomatic and may be found incidentally on computed tomography (CT) imaging of the chest done for other reasons. There is no uniform definition for the severity or degree of PE. The definition can be either anatomic or physiologic. The physiologic one is preferred for treatment decision making, as it is a better predictor of mortality. Any PE that causes hemodynamic instability (hypotension) is referred to as *massive PE*. *Submassive PE* is the term for PE associated with normal arterial blood pressure but right ventricular dysfunction that may be defined by electrocardiographic, echocardiographic, or CT criteria. Of note, in European guidelines (ESC 2014) PE with hypotension is also referred to as high-risk PE, whereas PE with a high pulmonary embolism severity index score and signs of right ventricular dysfunction is called intermediate risk, further distinguished as intermediate-high and intermediate-low risk based on the presence of 2 or 1 features of right ventricular dysfunction, respectively. Patients without additional risk factors are then assessed as low risk.

In children, the clinical presentations are similar, but for CVAD-associated VTE, loss of CVAD patency is a frequent earlier sign. Stroke secondary to paradoxical emboli can also be the primary presentation in children with right-to-left shunts, such as those with congenital heart disease or neonates with patent foramen ovale. Children often do not present with any acute symptoms, but rather long-term symptoms, including prominent collateral circulation in the skin over the related vessels, repeated loss of CVAD patency, repeated requirement for CVAD replacement, loss of venous access, CVAD-related sepsis, chylothorax, chylopericardium, and postthrombotic syndrome. The clinical presentation of cerebral sinus vein thrombosis is often very nonspecific. In neonates, seizures and lethargy

are frequent, and focal neurologic deficits rare. Headaches, seizures, cranial nerve palsies, and visual disturbances are more common in older patients. Intra-abdominal thromboses, such as mesenteric or splenic vein thrombosis, are even more nonspecific in their clinical presentations, with pain being the most common feature. Hepatic vein and portal vein thrombosis may present with signs of liver impairment or portal hypertension, respectively.

Pathophysiology of thrombosis

Thrombosis, defined as excessive clotting, has 3 main causes, referred to as Virchow's triad: reduced blood flow (stasis), blood hypercoagulability, and vascular wall abnormalities. Under normal circumstances, if blood vessel integrity is interrupted, coagulation takes place and a blood clot forms to prevent excessive bleeding. On the other hand, blood in the intact vasculature is kept in a fluid state by multiple endogenous antithrombotic factors that include normal endothelium and anticoagulants. These natural anticoagulants, such as antithrombin (AT), protein C, and protein S, prevent excess thrombin formation. Once a thrombus has formed, its growth is limited by clot lysis, which eventually leads to thrombus resolution.

VTE is a typical multicausal disorder, with more than 1 factor (genetic or environmental) needed for thrombosis to occur. A pathophysiologic model suggests that each individual has a baseline (or background) thrombosis risk that increases with age (Figure 9-1A). Transient risk factors, such as major surgery or estrogen therapy, temporarily increase a person's thrombosis risk, but the threshold of thrombosis formation often is not reached (Figure 9-1B). Most people, therefore, never develop symptomatic VTE. However, the individual with a higher baseline thrombosis risk, such as a known or unknown inherited or acquired intrinsic predisposition to clotting (thrombophilia), may cross the thrombosis threshold while exposed to a transient risk factor and thus present with symptomatic VTE (Figure 9-1B).

In general, venous thrombosis is caused by disturbances in the plasma coagulation system with platelet participation playing a proportionately minor role; whereas in arterial thrombosis platelets play the predominant role, with some participation of the plasma coagulation system. This paradigm helps explain why coagulation protein abnormalities, such as factor V Leiden (FVL), the prothrombin 20210 mutation, and deficiencies of protein C, protein S, and AT are associated with an increased risk of VTE but have not been linked consistently to a higher likelihood of arterial events, such as myocardial infarction or stroke. Thrombus formation in the cardiac ventricles and atria

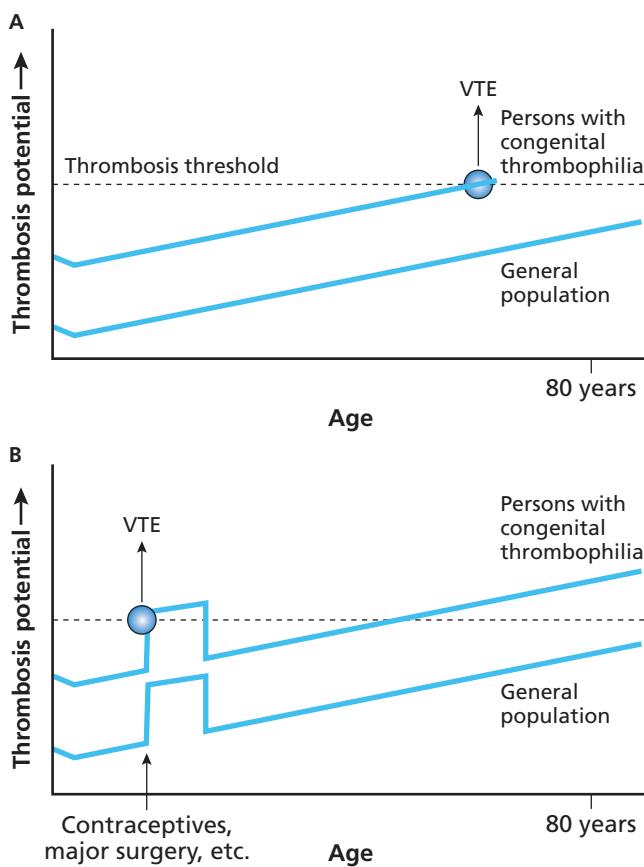


Figure 9-1 Threshold model of thrombosis risk. Modified from Rosendaal FR. *Lancet*. 1999; 353: 1167–1173.

often is caused by stagnant blood flow in dyskinetic, or aneurysmal parts of the heart chambers or in fibrillating atria. These intracardiac thrombi arise in a low-flow environment and are thus pathophysiologically thought to be similar to the thrombi that lead to venous thrombosis, even though there is no consistent relation with inherited thrombophilia or antiphospholipid syndrome.

Arterial clots usually form in areas of atherosclerotic vascular damage. The events leading to atherosclerosis—mainly lipid disturbances, oxidative stress, and inflammation—have been relatively well studied. The composition and vulnerability of plaque, rather than the severity of stenosis, are the most important determinants for the development of acute arterial ischemic syndromes. Disruption of the fibrous cap or endothelium overlying an atheromatous plaque exposes collagen and tissue factor to the circulating blood, leading to platelet adhesion and aggregation and local thrombin formation, with subsequent partial or complete vessel occlusion.

In general, the hemostatic system in neonates is a balanced physiologic system, despite low concentrations of

plasma coagulation proteins with prolonged prothrombin time and partial thromboplastin time, counterbalanced by physiologically decreased levels of natural coagulation inhibitors. The coagulation system of children evolves with age, with marked physiological differences in the concentration of the majority of blood clotting proteins, a concept known as “developmental hemostasis.” Notably, there is evidence that children are protected from thrombosis from a number of different perspectives. Patients with congenital AT, protein C, or protein S deficiencies, or with activated protein C resistance (APC resistance) may present early in life, but usually do not present with thrombosis until late teenage years or even later. In addition, VTE secondary to acquired risk factors occurs considerably less frequently in children compared to adults. Furthermore, children prior to puberty may undergo abdominal or trauma surgery without anticoagulant prophylaxis because secondary thromboses are rare. Apart from neonates, who are more susceptible to coagulation imbalances and therefore have a higher risk of developing provoked venous thrombosis, the low absolute risk of VTE in children as compared to adults suggests the presence of protective mechanisms.

Diagnosis of VTE

Since VTE is confirmed by objective testing in a relatively small percentage of patients presenting with possible DVT/PE, several clinical scoring systems (eg, Wells, Oudega, Hamilton, Geneva) have been validated in adults; by defining the pretest probability of disease, these scores help determine which diagnostic tests are most appropriate. Selected whole blood or plasma D-dimer tests are well evaluated and useful in the diagnostic workup for DVT and PE. In outpatients with a low pretest probability for DVT or PE, a negative test with a sensitive D-dimer assay reliably excludes VTE, and no further imaging study is needed. Outpatients with a low pretest probability for DVT or PE and a positive D-dimer test, and any patient with moderate or high pretest probability for DVT or PE, needs to undergo imaging studies. Algorithms with adjustments of D-dimer threshold levels that prompt imaging studies have recently been validated, either based on age (“Age-ADJUST”) or presence of specific items of the clinical prediction score (“YEARS”). The generalized application of D-dimer testing, however, is limited by the large number of different assays available, some highly sensitive and others less sensitive; the increase in baseline values with age; and a lack of standardization of assays. Because clinicians often are not aware of the type of D-dimer assay used by their laboratory or the predictive value of the particular assay available to them, reliance on D-dimer results for clinical decision making for the exclusion of VTE can be unwise,

unless the test has been validated locally. In children, the D-dimer test as a diagnostic tool for VTE has not been well studied and available evidence does not support its use. The D-dimer test is of limited diagnostic utility in a variety of conditions (eg, pregnant patients, patients with cancer, sickle cell disease) where it is known to be elevated at baseline. However, patients with cancer have been included in management studies and although the efficiency of the algorithm diminishes, still in some patients it can help to exclude the diagnosis without further imaging tests.

Venous compression ultrasound (CUS) is the most widely used imaging study to look for DVT of the legs. Sensitivity and specificity of the test is operator dependent, especially for distal lower extremity DVT, and an experienced ultrasound technician or physician is key in obtaining reliable results. It can be challenging, even for an experienced operator, to distinguish between acute vs chronic thrombus solely based on CUS. Magnetic resonance (MR) venography of leg or pelvic veins is a sensitive test to detect DVTs, but it is expensive and not widely available. Imaging with MR or CT venography may be necessary for upper-extremity DVT, particularly catheter-related events, because ultrasound may miss occlusion within the superior vena cava and brachiocephalic and subclavian veins due to interference of the clavicles and ribs. Ultrasound is the most common modality used in children; however, its validity should be carefully considered. The low pulse pressure in premature newborns likely makes CUS more difficult to interpret. Similarly, the presence of CVADs makes compressibility difficult to assess, which greatly reduces the sensitivity of CUS. In the upper system, compressibility is not possible for veins below the clavicle and the PAARKA study demonstrated ultrasound to have a sensitivity of 20% for intrathoracic thrombosis; yet diagnosed jugular thrombi that were missed on venography.

To diagnose PE, several imaging modalities exist: ventilation/perfusion (V/Q) scanning, chest CT pulmonary angiography (CTPA; also known as spiral CT, helical CT, or PE-protocol CT), chest MR angiography, and conventional intravenous contrast pulmonary angiogram. The V/Q scan is a well-validated imaging study. CTPAs have replaced V/Q scans as the diagnostic method of choice because they are easier and faster to perform and have good performance characteristics. Conventional intravenous contrast pulmonary angiography, once considered the gold standard for the diagnosis of PE, now is rarely done because the test is invasive and not widely available. There is ongoing debate about the clinical significance of isolated tiny pulmonary artery filling defects that can be

seen on chest CT scans (subsegmental PE), and the clinical relevance is likely dependent upon underlying conditions (eg, cancer) and clinical situation (hospitalized vs performed for cancer screening, for instance). There are a number of potential difficulties with interpreting V/Q scans in children. In children, following specific cardiac surgeries such as Fontan surgery, total pulmonary blood flow is not assessed by isotope injected into an upper limb. Injection into both upper and lower venous systems is required, but even then, the impact of intrapulmonary shunting may make interpretation difficult. In addition, there are concerns about the safety of perfusion scanning in children with significant right-to-left cardiac shunts, as likely significant amounts of macroaggregated albumin lodge in the cerebral circulation, and the impact of this is unknown. Repeated CTPA may cause significant radiation exposure to breast tissue in young female patients.

Acute therapy of VTE

Patients with acute VTE need to be anticoagulated to prevent the extension of thrombus and decrease mortality. Direct oral anticoagulants are preferred over vitamin K antagonists (VKAs) because of their lower risk of intracranial and fatal bleeding in patients without contraindications and without cancer. Treatment of cancer-associated VTE is discussed in a separate paragraph. Apixaban (higher initial dose for 7 days) and rivaroxaban (higher initial dose for 21 days) can be used to treat acute DVT or PE without prior parenteral therapy. Subcutaneous low-molecular-weight heparin (LMWH) or fondaparinux dosed based on body weight, and intravenous unfractionated heparin (UFH) with activated partial thromboplastin time (aPTT) monitoring and dose adjustments, are all effective and acceptable treatment options and need to be given for at least 5 days (overlapping with warfarin until the international normalized ratio [INR] is ≥ 2.0 on 2 consecutive occasions, or *prior to* starting dabigatran or edoxaban, if 1 of these agents is used). In high-risk PE patients who may require thrombolysis, UFH is preferable to direct oral anticoagulants (DOACs), LMWH, or fondaparinux because it has a shorter half-life and easily can be dose-adjusted, discontinued, or reversed with protamine. In selected patients with extensive acute femoral or iliac DVT with symptom duration of < 14 days and low bleeding risk, catheter-directed thrombolysis with or without mechanical thrombus fragmentation and aspiration can be considered to reduce acute symptoms. However, the recently reported ATTRACT trial showed that pharmacomechanical thrombolysis of femoral or iliac DVT, in addition to standard anticoagulation, leads to faster resolution of symptoms and improved canalization rates, but does not improve the primary out-

come measure of postthrombotic syndrome (PTS) after 2 years.

Thrombolytic therapy in PE is indicated for massive life-threatening PE (ie, PE with hypotension due to right ventricular dysfunction). However, patients with submassive or intermediate-high risk PE (ie, those without hypotension but with right ventricular dysfunction) do not convincingly benefit from thrombolytic therapy, due to the increased risk of major (including intracranial) bleeding. These patients require close monitoring, as “rescue thrombolytic therapy” seems beneficial in patients who develop cardiovascular collapse after initially being treated with anticoagulant therapy alone. Also, long-term (approximately 3 years) follow-up does not show benefit of thrombolysis in terms of persistent symptoms or complaints in patients with submassive or intermediate-high risk PE. If thrombolytic therapy is given to a patient with PE, it is recommended that it be given systemically via a peripheral vein and with short infusion time, such as 2 hours. Catheter-directed thrombolysis for massive PE using lower doses of tissue plasminogen activator (tPA) is available in some centers, but there is no randomized controlled evidence showing that this is more effective or safer than systemic thrombolysis.

Outpatient management of patients with DVT and selected low-risk patients with PE has been shown to be safe, feasible, cost effective, and (if possible) is the preferred treatment of choice. Hospital admission is appropriate if either the patient is too sick to be managed at home or if social and financial circumstances make this the safer and more feasible option. LMWH, UFH, and warfarin remain the mainstays of antithrombotic therapy in children. Some centers use fondaparinux. DOACs remain in clinical trials but due to lack of safety and efficacy data, should not be used outside of the trial scenario until trials have been completed.

Patients with cancer merit special consideration, as cancer can be considered an acquired thrombophilic condition, as discussed in the paragraph on acquired thrombophilia.

Patients with cancer have a strongly increased risk of VTE. Furthermore, they are at high risk of recurrence despite the use of therapeutic anticoagulants. LMWH (full therapeutic for the first 4 weeks, and 75% of therapeutic dose thereafter) has been shown to be more effective than VKAs in preventing recurrences in these patients and has been the recommended treatment for the first 6 months after the acute VTE (thereafter, it is unknown because no studies have compared LMWH with VKAs), if feasible from a financial and insurance reimbursement point of view. The Hosukai-Cancer VTE study showed that the factor Xa inhibitor edoxaban was noninferior to LMWH in the treatment of cancer-associated thrombosis (CAT), with a

composite endpoint of recurrent VTE or major bleeding. An apparent greater efficacy was balanced against an increase in major bleeding, particularly from the gastrointestinal tract. The smaller Select-D study with rivaroxaban, from which patients with upper gastrointestinal tract cancers were excluded, showed similar results. A similar trial comparing apixaban with LMWH is currently ongoing. It remains uncertain if patients with all types of cancers have the optimum risk-benefit balance. The current guidelines have not yet been updated after these 2 trials, but it is to be expected that the use of DOACs in this population will rapidly increase.

Recurrent VTE despite anticoagulant treatment

Treatment with therapeutic anticoagulants (ie, VKA, DOAC or therapeutic-dose LMWH), reduces the risk of extension or recurrence by 80% to 90%. In patients who fail anticoagulation, the clinician should remain vigilant for evidence of cancer, antiphospholipid syndrome (APS), or an anatomic cause of thrombosis. Clearly, issues with adherence should always be considered.

If a patient presents with recurrent VTE despite therapeutic anticoagulation, treatment options are either to increase the target INR (for patients on VKA), increase LMWH dose by 25%, add another anticoagulant, or switch to another anticoagulant (particularly from VKA to LMWH). There are no robust data on the comparative effectiveness of the different anticoagulants or strategies in this setting.

Further pediatric considerations

The target therapeutic ranges for all anticoagulants are extrapolated from adult data, despite the known age-related differences in pharmacokinetics. LMWH is the preferred anticoagulant in children for the treatment of VTE because of its predictable pharmacokinetics, lack of interference with diet, and easy availability of anti-Xa assays for its monitoring. However, the need for twice-daily injections, and concerns related to bone density, limit its long term use. The pediatric doses are calculated according to age and weight of the patient, as both influence the volume of LMWH distribution. Young infants (age <2 months) require higher doses of LMWH and UFH. Oral VKA therapy is a good option in children, provided there is adequate expertise and resources to support an outpatient anticoagulant management service that includes education of both child and parents. VKA management in young infants could be challenging for several reasons: (i) physiologic reduction of vitamin K-dependent coagulant proteins; (ii) excessive sensitivity to VKAs in breastfed infants; (iii) resistance to VKA therapy due to vitamin K intake in infant formula; (iv) lack of availability of liquid

formulation in many countries; (v) vascular access issues for INR monitoring. The use of home INR monitoring using capillary samples greatly increases the acceptability of VKA in all age groups. The experience of using fondaparinux is limited in children, but dosing regimens are available in children older than 1 year of age. When fondaparinux is used, it is monitored with anti-factor Xa assays. The 2012 American College of Chest Physicians (ACCP) guidelines provide details on dosing regimens and monitoring for specific anticoagulants. At this time, there is no evidence to suggest an advantage of local over systemic thrombolytic therapy in children with thrombotic complications. In addition, the small vessel size in children may increase the risk of local vessel injury during catheter-directed therapy. The theoretical advantages of catheter-directed thrombolysis include the ability to deliver low doses of thrombolytic agent directly into the thrombus. Local therapy may be appropriate for catheter-related thromboembolism when the catheter is already in situ. There are more recent small case series reporting catheter-directed thrombolysis in children.

Adjunctive therapies

Inferior vena cava filters

A clear indication for an inferior vena cava (IVC) filter exists only when a patient has acute proximal leg vein thrombosis or PE *and* has an absolute contraindication to anticoagulation. It is not clear whether an IVC filter is beneficial in the patient with recurrent pelvic or proximal leg DVT despite therapeutic anticoagulation. Recent reviews suggest that IVC filters should not be used for primary VTE prevention in patients with trauma or undergoing major abdominal or pelvic surgery. When an IVC filter must be placed, a retrievable filter should be used. Retrievable filters can be left in place for weeks but should be removed as soon as clinically possible and only remain permanently if absolutely necessary. For patients with acute VTE who have an IVC filter inserted as an alternative to anticoagulation, anticoagulant therapy should be initiated or resumed as soon as the patient's risk of bleeding permits. The presence of an IVC filter increases a patient's risk for recurrent DVT and confers a risk of caval vein thrombosis. When making a decision on the length of anticoagulant therapy in a patient with a permanent IVC filter, the presence of the IVC filter should be viewed as a risk factor for recurrent VTE.

Venous stents

May-Thurner syndrome is the term used for the chronic compression of the left common iliac vein between the

overlying right common iliac artery and the fifth lumbar vertebral body posteriorly. Varying degrees of vein narrowing with this anatomic variant are common in the general population. If May-Thurner syndrome is demonstrated on venography or magnetic resonance imaging (MRI) in the patient with left-leg femoral or iliac DVT who successfully has received thrombolytic therapy, correction of the stenosis using balloon angioplasty and stenting can be considered, although there is no high-quality evidence that such interventions reduce the risk of recurrent VTE or PTS.

Although there are no clinical trials to determine their efficacy, venous stents are sometimes placed in various locations of the venous system, either in the acute DVT context of catheter-directed thrombolysis, or to alleviate severe postthrombotic syndrome—most commonly into the left common iliac vein for May-Thurner syndrome, the right and left pelvic veins for postthrombotic vessel narrowing and scarring, and the superior vena cava and central arm veins in central venous catheter-associated strictures. Of note, the best long-term management of patients who have venous stents is not known, due to a lack of high quality prospective studies examining their long-term patency with and without antiplatelet drugs or anticoagulants. Because stents are foreign bodies in the venous system and may lead to flow disturbances, it is possible that they have some prothrombotic risk. In addition, endothelial cell proliferation within stents is known to occur, potentially leading to stent stenosis and occlusion. In view of the limited data available, it may be best to view the presence of a venous stent as a potential risk factor for recurrent VTE. After venous stent placement, it may be reasonable to keep a patient on anticoagulants for 3 months and then make an assessment on the need for long-term anticoagulation vs no further anticoagulation based on a comprehensive assessment of the patient's risk factors for recurrent VTE and bleeding.

Duration of anticoagulant therapy

The risk of recurrent VTE depends on the presence of risk factors, either transient or persistent, during the first VTE. In patients with a VTE secondary to a major transient (reversible) risk factor, the risk of recurrence is low. Therefore, time-limited anticoagulation for 3 months with a DOAC or VKA is recommended. For patients with unprovoked proximal leg DVT or unprovoked PE in whom risk factors for bleeding are absent and for whom good anticoagulation control is achievable, long-term (extended) anticoagulation therapy should be strongly considered. Unselected patients who stop anticoagulants after some initial (eg, 3- to 12-month) period of treatment for unpro-

voked VTE have a 3-year recurrence rate of 20% to 30%; at 5 years these numbers are approximately 40% and 50%. Several parameters can be used in an effort to individualize the risk of recurrence (Table 9-1). This, along with the patient's bleeding risk factors and personal preferences, should be used to help the patient make an informed decision about whether to continue therapy (Figure 9-2). When extended anticoagulation therapy is chosen, the risks, benefits, and burdens should be reevaluated periodically (eg, once a year). For patients with a first episode of unprovoked distal (ie, below the trifurcation of the popliteal vein) leg DVT, 3 months of anticoagulant therapy is recommended.

To aid decision making about which patients continue or discontinue anticoagulation, risk scores have been created using data from VTE trials in which the rate of re-

current VTE was recorded and subgroup analyses were performed. Male sex is associated with a higher risk of recurrence; in women, the absence of postthrombotic syndrome, a low D-dimer (measured after stopping anticoagulation for 4 weeks), and possibly a body mass index of $<30 \text{ kg/m}^2$ predict a lower risk of recurrence. Patients with unprovoked VTE who have access to one of the DOACs may be especially good candidates for extended anticoagulation because these medications not only are more convenient than VKAs but also provide excellent protection against recurrent thrombosis, with a comparatively small increase in the risk of major bleeding. Low-dose apixaban (2.5 mg po BID) or rivaroxaban (10 mg po daily) decreases the incidence of recurrent VTE after the use of higher doses for 6 months, is more efficacious than low-dose aspirin at decreasing recurrent VTE and is associated with a low overall risk of bleeding. It is important to note that the availability of these reduced dose anticoagulant regimens lowers the threshold for continuing secondary prophylaxis, particularly in patients with VTE provoked by minor transient or persistent risk factors, or those with recurrent VTE provoked by major risk factors. Patients who choose to discontinue anticoagulation or have no access to DOACs but are still at some risk for recurrent VTE should be informed that daily low-dose aspirin is relatively safe and appears to reduce the likelihood of DVT/PE by about 30%. Hence, for some patients, low-dose aspirin may achieve the best balancing of risk, benefit, and cost considerations.

Whether thrombophilia testing should be performed to determine the duration of anticoagulant treatment for a patient with unprovoked VTE is discussed later in this chapter.

Duration of anticoagulation in children is essentially extrapolated from adult data. Provoked VTE is usually treated for 3 months or until the risk factor (eg, CVAD) is removed. Anecdotally, many clinicians treat neonates or young infants for 6 weeks only if there is total radiological resolution of thrombus, and the validity of this approach is addressed in the ongoing KIDS DOTT trial. The optimal duration of therapy for unprovoked VTE is unknown and the impact of anticoagulation on the patient's lifestyle and mental health, as well as patient preferences, are significant considerations.

Postthrombotic syndrome

PTS may be caused by several factors, including incompetent venous valves damaged by the thrombus, associated inflammatory mediators, and impairment of venous return due to residual venous obstruction from incompletely cleared thrombus. Fewer than 5% of DVT patients

Table 9-1 Considerations when discussing time-limited vs long-term anticoagulation therapy in adult patients with *unprovoked* VTE

Clinical factors that favor extended anticoagulant therapy	
History of recurrent VTE	
Male sex	
Patient had a PE, not a DVT	
D-dimer on anticoagulant therapy elevated at 3 or 6 months*	
D-dimer elevated after having been off anticoagulants for 4 weeks*	
Obesity	
Older age	
Persistent underlying risk factor such as active cancer or inflammatory bowel disease	
Anticoagulant therapy well tolerated (with good INR control, if on VKA)	
Little or no impact of anticoagulant therapy on patient's lifestyle	
Patient's preference is to continue treatment	
Patient has a known, strong thrombophilia (either congenital or acquired)	
Factors favoring limited duration of anticoagulation	
Female sex	
Distal DVT only	
D-dimer negative after having been off anticoagulation for 4 weeks (most relevant to women)	
No signs of PTS (most relevant to women)	
Occurrence of bleeding complications or significant risk for bleeding	
Patient's preference is to be off anticoagulants	

DVT, deep vein thrombosis; INR, international normalized ratio; PE, pulmonary embolism; PTS, postthrombotic syndrome; VKA, vitamin K antagonist; VTE, venous thromboembolism.

*Cutoff is assay specific (not all D-dimer assays have been studied for this purpose; clinicians should check with their local laboratory).

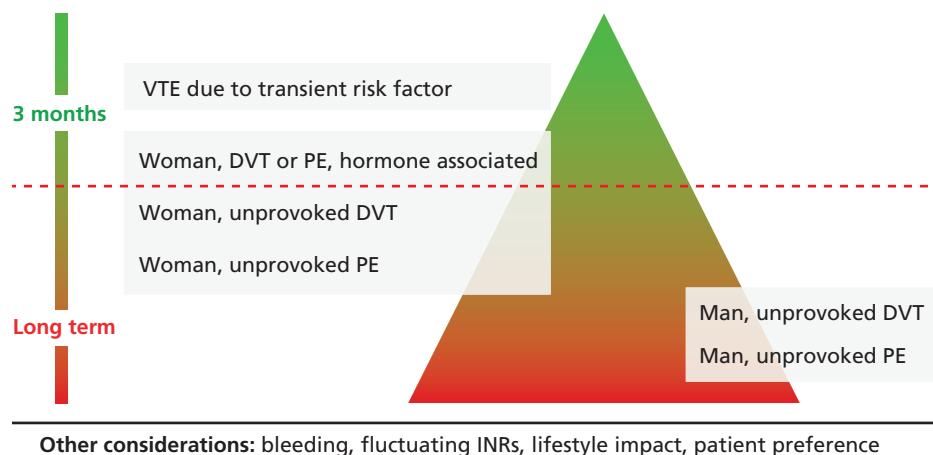


Figure 9-2 Management strategy regarding length of anticoagulation therapy decisions in patients after a first episode of provoked or unprovoked proximal VTE. DVT, deep vein thrombosis; INR, international normalized ratio; PE, pulmonary embolism.

develop severe PTS (sometimes referred to as postphlebitic syndrome), in most cases within 1 to 2 years of the acute DVT. However, up to 30% of patients experience symptoms of mild to moderate PTS. Symptoms and signs include chronic extremity swelling, pain, heaviness, fatigue, paresthesias, skin induration, dryness, pruritus, erythema and chronic dark pigmentation; and in severe cases, skin ulcers. The risk for developing postthrombotic syndrome had previously been found to be decreased by wearing graduated compression stockings (40 mm at ankle, 30 mm at midcalf) for 2 years after an acute DVT, but a recent placebo-stocking randomized controlled trial showed no impact of this intervention on the risk of PTS. Another recent trial showed that in patients who have no symptoms of PTS 6 months after DVT, a longer duration stocking does not reduce PTS incidence. Treatment options for patients with postthrombotic syndrome are limited. Compression stockings should be worn if they provide symptom relief. In patients with significant leg swelling, imaging of leg veins with Doppler ultrasound and of the pelvic veins with CT or MR venography can be considered to evaluate for focal pelvic vein obstruction or narrowing due to May-Thurner syndrome or postthrombotic scarring. Although these sorts of anatomic abnormalities might be amenable to pelvic vein angioplasty and stenting, there is no high-quality evidence to support the efficacy or safety of such interventions in this challenging clinical situation. Finally, a home compression pump with compression sleeve for the affected leg can be considered for patients with significant symptoms. In children, the challenge is often to get appropriately fitted stockings or sleeves for use in upper limbs. The next challenge is to get

the children to wear them. There are no effective studies and management is extrapolated from adults.

Pulmonary hypertension

Pulmonary hypertension due to VTE, termed *chronic thromboembolic pulmonary hypertension* (CTEPH), is defined as an elevated mean pulmonary artery pressure of >25 mm Hg (without evidence of left-heart failure) and occurs in 1% of patients with acute PE after 6 months. One study has reported CTEPH in 3.8% of PE patients after 2 years; the authors' experience suggests that clinically apparent CTEPH may be less common. CTEPH can be the result of a single episode of PE that did not resolve appropriately, or the result of recurrent episodes of PE. The patient who experiences chronic shortness of breath or significant generalized malaise after PE should be evaluated for pulmonary hypertension. A formal 6-minute walk test with pre- and postexercise pulse oximetry measurements is appropriate. It is important to realize that chest CT angiogram findings may be minimal with chronic distal PE. A V/Q scan is probably more sensitive for chronic PE. Echocardiography can be used to estimate pulmonary artery pressure. Right-heart catheterization with pulmonary artery pressure measurements then defines the degree and etiology of hypertension, and pulmonary arteriography allows assessment of whether potentially curative pulmonary endarterectomy is indicated, although only performed in specialized centers. Long-term anticoagulant therapy is indicated. Pharmacologic therapy specific for pulmonary hypertension, such as bosentan (an endothelin receptor antagonist), can be considered in the inoperable patient.

Primary prevention of VTE

Prophylaxis against VTE should be considered in every hospitalized patient based on an individual patient's risk stratification. Detailed prophylaxis guidelines for all types of patients have been published in the medical literature, most notably the 2012 ACCP guidelines. Formal VTE prophylaxis protocols should be in use in all hospitals. The most convincing evidence of net benefit from VTE prophylaxis comes from surgical populations.

If anticoagulation for VTE prophylaxis is appropriate, several options are available: (i) LMWHs at once- or twice-daily intervals; (ii) fondaparinux once daily; (iii) VKAs; or, in patients undergoing total knee or hip replacement, (iv) apixaban or rivaroxaban can be used. FDA-approved indications vary between the different pharmacological options. Although there is evidence that aspirin and other antiplatelet agents provide some protection against VTE in hospitalized patients at risk, they are probably less effective than other pharmacologic methods of VTE prophylaxis. In the specific setting of total hip or knee replacement, a recent randomized trial showed, after an initial 5 days of 10 mg of rivaroxaban, noninferiority of low-dose aspirin compared to rivaroxaban for an additional 9 (after knee replacement) to 30 days (after hip replacement) for post-operative VTE. Prophylaxis may be given only during the hospitalization or, if the VTE risk persists after discharge home, for an extended period of time. The net benefit and cost effectiveness of postdischarge prophylaxis (up to 5 weeks), are well established in patients after hip fracture, hip replacement, total knee replacement, and major cancer surgery.

Mechanical methods of prophylaxis with graduated compression stockings or intermittent pneumatic compression devices typically are recommended for patients who are at high risk for bleeding or as an adjunct to anticoagulant-based prophylaxis. They often are not suggested as a first choice for primary prevention because they have been studied less intensively than anticoagulant-based methods.

Pediatric consideration

There is growing concern about rising prevalence of VTE in hospitalized children, but the role of pharmacological thromboprophylaxis in preventing hospital-acquired CVAD-related thrombosis is controversial. While CVAD is the most common risk factor for VTE, it is estimated that less than 2% of children with CVAD get symptomatic VTE. No study has demonstrated successful risk reduction of short- to moderate-term CVAD-associated VTE with pharmacological prophylaxis. Uniform prophylaxis for CVADs, even in pediatric cancer populations, cannot

be recommended. Better risk stratification algorithms and the risk-benefit ratios of therapy need to be determined.

The studies performed to date of primary prophylaxis of hospitalized children suggest that prepubertal hospitalized children rarely require thromboprophylaxis. Postpubertal children with multitrauma, sepsis, or hypotension may require thromboprophylaxis in the presence of additional risk factors such as obesity (>95th percentile or body mass index of >30); oral contraceptive pill; dehydration; estimated length of stay >4 days; family history of VTE; known thrombophilia and CVAD. Similarly, in postpubertal children having prolonged surgery, early ambulation, calf compression, and the use of elastic compression stockings are likely adequate unless there are additional risk factors as outlined previously and strict bed rest enforced for >4 days. For these in-hospital children, once-daily enoxaparin is most commonly used if pharmacological prophylaxis is required.

Anticoagulation prophylaxis with oral VKA therapy is currently recommended for children receiving long-term home total parenteral nutrition on the basis of small numbers of cohort studies.

Much more work has focused on primary prevention in pediatric cardiac surgical populations. Modified Blalock-Taussig shunts, and Fontan procedures in particular, have been the focus of a number of studies. Cardiomyopathies, pulmonary hypotension, and prosthetic cardiac valves are all common indications for primary prophylaxis. While there is general agreement that prophylaxis is worthwhile and any prophylaxis reduces the thrombosis risk, the optimal agent, dose intensity, and duration remain unclear.

Superficial thrombophlebitis and unusual site venous thromboses

Superficial thrombophlebitis

Superficial thrombophlebitis in the legs refers to the peroneal, posterior tibial, and saphenous veins. In the upper extremities it refers to antecubital, basilic, and cephalic veins. Risk factors concur with those for VTE; and in addition, varicose veins, intravenous catheters or phlebotomy, or septic thrombophlebitis with infections are commonly associated. Superficial vein thrombosis also occurs in association with thrombangiitis obliterans (Buerger disease) and Behcet disease. The term *Trousseau syndrome* often is used for migratory thrombophlebitis in patients who subsequently are diagnosed with cancer, but the term is not well or uniformly defined.

Extension of superficial thrombophlebitis into the deep venous system of the leg occurs in about 1 in 6 patients with extensive superficial thrombophlebitis and often is present at time of diagnosis. To rule out concomitant

DVT or extension, CUS should be performed at diagnosis, and follow-up CUS should be considered in patients for whom anticoagulation is not prescribed.

Patients with extensive or recalcitrant superficial thrombophlebitis benefit from a short course of out-of-hospital anticoagulant therapy, such as 6 weeks of subcutaneously administered fondaparinux, low-dose DOAC, or LMWH. Prophylactic dose fondaparinux (2.5 mg daily) for 45 days, in comparison to placebo, has been shown to reduce the risk of DVT and superficial vein thrombosis (SVT) extension and SVT recurrence. The number needed to treat to prevent 1 clinically important event is 20, and for symptomatic DVT or PE 88, which has led to debates regarding the cost effectiveness of routinely anticoagulating patients with superficial thrombophlebitis strategy. A recent randomized controlled trial that compared 10 mg rivaroxaban with 2.5 mg fondaparinux showed that fondaparinux was associated with a nonstatistically significant reduction of symptomatic VTE, DVT, recurrence of SVT, mortality, clinically relevant nonmajor bleeding, serious adverse events, or adverse effects of treatment compared with rivaroxaban. For LMWH, there is only low-quality evidence regarding the optimal dosing (full dose, intermediate dose, or prophylactic low dose) and the duration of therapy, without showing a reduction in symptomatic VTE. Thrombophlebitis that is not very extensive (ie, <5 cm in length and not close to the deep venous system) requires only symptomatic therapy, consisting of analgesics, anti-inflammatory medications, and warm or cold compresses for symptom relief, although the evidence is very limited and does not inform clinical practice about the effects of these treatments in terms of VTE.

Upper-extremity DVT (and catheter-related thrombosis)

The superficial veins of the arm include the antecubital, cephalic, and basilic veins. The deep venous system includes the brachial vein, which becomes the axillary vein, followed by the subclavian and brachiocephalic veins, and finally the superior vena cava. Upper-extremity DVTs make up 1% to 4% of all DVT in adults (compared to the majority of pediatric DVT due to the frequency of CVAD placement in the upper venous system). In adults, roughly 80% are secondary to central venous catheters and cancer, and 20% are primary events; however, these data depend largely on which patient population is studied. Doppler ultrasound (sensitivity 78% to 100% and specificity 82% to 100%), contrast venography (gold standard), and CT or MR venography are the tools used to diagnose upper-extremity thrombosis. In adults, the risk of PE with upper-extremity DVT is not well defined and depends on the

modality used to detect it, but seems to be low (especially if the clot is catheter associated). Postthrombotic syndrome is common; residual thrombosis and axillosubclavian vein thrombosis appear to be associated with a higher risk of upper-extremity PTS, whereas catheter-associated DVT may be associated with a lower risk. These associations are less clear in children.

Management for DVT of the upper extremity consists of the following: (i) LMWH, UFH, or fondaparinux in the acute setting; (ii) continued anticoagulation for at least 3 months for unprovoked DVT or catheter-associated DVT; and (iii) no catheter removal in patients with DVT associated with a central venous catheter if the catheter is functional and still needed. In children, especially those with right-to-left shunts, a period of anticoagulation prior to catheter removal is frequently advocated to reduce the risk of paradoxical embolus at the time of removal. A DOAC is likely effective in upper-extremity DVT and although these agents have not been studied in catheter-associated thrombosis, the results of large, prospective randomized controlled trials of DOACs in the treatment of VTE support their consideration in the acute and long-term treatment of noncancer patients with catheter-related upper-extremity DVT. Decisions about duration of therapy for upper-extremity DVT usually are based on information extrapolated from studies of patients with lower-extremity DVT or PE. For catheter-associated DVT, a brief period (4 to 12 weeks) of anticoagulation after catheter removal is likely sufficient. There is little or no direct evidence to support any particular duration of anticoagulant therapy after a first unprovoked (or catheter-associated) upper-extremity DVT.

Upper-extremity DVTs may be due to thoracic outlet syndrome, also referred to as effort thrombosis, thoracic outlet syndrome, or Paget-Schroetter syndrome. This is due to compression of the axillary vein by pressure from the clavicle, an extra rib, or enlarged or aberrantly inserted muscles, often provoked or potentiated by abduction of the arm and repetitive arm movements. Younger athletes are often affected. There is no uniform approach to treatment of these patients. Management options include anticoagulation, thrombolytic therapy, angioplasty with or without stent placement, thoracic outlet surgery with rib or soft tissue resection, and surgical resection of the focally narrowed vein with vein reconstruction. Individual treatment decisions need to be made, and a team approach that includes vascular medicine, vascular surgery, and interventional radiology may be appropriate.

Hepatic vein thrombosis

Hepatic vein thrombosis, also referred to as Budd-Chiari syndrome, has varied clinical presentations, ranging from

asymptomatic to fulminant liver failure. A cause can be identified in approximately 84% of patients. Similar to other venous thromboembolic disorders, Budd-Chiari syndrome also often has a multifactorial etiology. Most patients (84%) have at least 1 thrombotic risk factor, and many (46%) have more than 1 risk factor; the most common being myeloproliferative neoplasms (MPNs) (49% of patients). Polycythemia vera accounts for 27% of cases; essential thrombocythemia (ET) and primary myelofibrosis are less prevalent causes. The *JAK2* mutation is present frequently in patients with the syndrome (29% of cases), even if no hematologic abnormalities suggestive of an MPN are present. This is discussed in detail in the “Thrombophilias” section in this chapter. Any of the inherited and acquired thrombophilias can contribute to the development of Budd-Chiari syndrome, as can estrogens and pregnancy. Paroxysmal nocturnal hemoglobinuria (PNH), although an uncommon disorder, can be detected in almost one-fifth of patients with Budd-Chiari syndrome.

The diagnosis is made by Doppler ultrasonography, contrast-enhanced CT scanning, or MRI. In the acute setting of fulminant thrombosis, thrombolytic therapy can be considered. Angioplasty of narrowed or occluded hepatic veins can be performed, shunt procedures may be required, and liver transplantation may be necessary. Anticoagulation is usually appropriate and often is given long term, typically with VKAs. INR monitoring may be problematic, however, because liver synthetic dysfunction may lead to a baseline elevation of INR even before VKA therapy. Alternative monitoring tests for VKAs, such as factor II or X activity, may have to be used. Also, treatment with LMWH, fondaparinux, or a DOAC instead of VKAs can be considered. Hepatic vein thrombosis is rare in children.

Portal vein thrombosis

Portal vein thrombosis (PVT) often is silent and may be discovered only upon evaluation of a variceal gastrointestinal bleed. It is associated with the inherited and acquired thrombophilias, the MPNs, *JAK2*-positive status without overt MPN, PNH, intra-abdominal neoplasia or inflammation, infection, trauma, and surgery.

It occurs in up to 26% of patients with cirrhosis of the liver. As with other venous thromboembolic disorders, multiple contributors often are identified. In a number of cases, no predisposing factor is found. In newborns, PVT most commonly occurs secondary to umbilical vein catheterization, with or without infection. The most common cause of PVTs in older children is postliver transplantation, although cases associated with intra-abdominal sepsis, splenectomy, sickle cell anemia, and the presence of antiphospholipid antibodies (APLAs) are reported. In

approximately 50% of children, an underlying etiology is not identified. In contrast to adults with PVT, liver function is usually normal in children. Diagnosis typically is made by Doppler ultrasonography. CT or MR venography also can provide evidence that PVT is present. Cavernous transformation of the portal vein reflects old PVT, as do collaterals in the porta hepatis. In the patient with acute PVT, extension of thrombus into the mesenteric veins may occur and lead to intestinal infarction and the need for surgical bowel resection. The patient with acute PVT typically is anticoagulated for at least 3 to 6 months to prevent progression of thrombosis. Regarding long-term anticoagulation therapy in these patients, as well as in patients with incidentally discovered PVT, the risk of bleeding has to be balanced individually against the risk of re-thrombosis. The net benefit of anticoagulation for a patient with asymptomatic, cirrhosis-associated PVT is uncertain. Patients with cirrhosis-associated PVT are at high risk of both bleeding and thrombotic events. Thus, management has to be tailored to the individual patient. The factors to be considered before long-term anticoagulation is prescribed include identification of the triggering factor for the thrombotic event, the extent of thrombosis, the presence of persistent prothrombotic factors, the extent of esophageal and gastric varices, the presence and degree of thrombocytopenia due to hypersplenism, and other risk factors for bleeding.

Mesenteric vein thrombosis

Venous drainage of the intestine is via the superior mesenteric vein (SMV) and inferior mesenteric vein (IMV) into the portal vein. The SMV drains the small intestine and ascending colon, whereas the IMV drains mostly the sigmoid colon. The transverse and descending colon can drain through the middle and left colic veins either into the SMV or IMV. SMV thrombosis, if diagnosed late, leads mostly to small bowel ischemic changes. The very rare IMV thrombosis may lead to ischemia in the sigmoid colon. Mesenteric vein thrombosis (MVT) may be caused by trauma, surgery, intra-abdominal infections, inflammatory bowel disease, pancreatic disease, and progression of PVT, but also may occur spontaneously, particularly in patients with inherited or acquired thrombophilias, MPNs, presence of the *JAK2* V617F mutation, and PNH. Symptoms are vague, often leading to a delay in diagnosis. Nonspecific abdominal pain is common, and nausea may be present. Gastrointestinal bleeding and peritonitis are seen when transmural ischemia has occurred. Symptoms may be present for days to weeks before a diagnosis is made, which often may occur only when the patient presents as a surgical emergency with ischemic bowel. The principal

cause of a high mortality rate in MVT is a delay in diagnosis. The surgical findings are typically those of a dusky but not frankly gangrenous intestine, unless full bowel-wall infarction already has occurred. Areas of viability of intestine are not as sharply demarcated as they are in arterial mesenteric ischemic disease. A mesenteric artery pulse is typically felt. Preoperative diagnosis is made by CT angiography. Doppler ultrasound may be diagnostic, but it is operator dependent and may have limited sensitivity, particularly in the obese patient. Once diagnosed, patients are managed with anticoagulation alone or in combination with surgical intervention. Most patients improve. Decisions on length of anticoagulant therapy depend, as with most of the other VTE disorders, on the triggers for the thrombotic episode and the presence of thrombophilias or other persistent underlying risk factors. Length of treatment is at least 3 months but may have to be long-term. The role of anticoagulation and/or antiplatelet therapy in the long-term secondary prevention of MVT in a patient with a confirmed MPN is not well established.

Splenic vein thrombosis

Because of the intimate anatomic contact of the splenic vein with the pancreas, the main causes of splenic vein thrombosis are pancreatitis and pancreatic malignancies. Similar to MVT, intra-abdominal problems (infection, surgery, and trauma) and thrombophilias also play a role in the etiology. Symptoms often are subtle, and the diagnosis is not infrequently a coincidental discovery on abdominal imaging studies done for other reasons. The need for, and length of anticoagulant treatment is not well defined, and depends on the triggering factors and the persistent underlying risk factors weighed against bleeding risk.

Cerebral and sinus vein thrombosis

Blood from the brain drains via cerebral and cortical veins into the dural sinuses that then drain into the internal jugular veins. Thrombosis of the cerebral, cortical, and sinus veins often is referred to as *cerebral sinovenous thrombosis* (CSVT). It occurs in 1 to 2 cases per 100,000 in the general population, about 3 times as often in women than in men, due to the strong association with sex-specific risk factors such as oral contraceptive use, pregnancy, and postpartum period. Unlike VTE at other locations, less than 10% of adults are older than 65. Approximately 80% of adults recover without functional disability, but early mortality is usually caused by transtentorial cerebral herniation due to large space-occupying lesions or generalized cerebral edema. Most neonatal CCSVT occurs during the first week of life and seizures are the most common presenting symptom. Altered consciousness and focal motor deficits

are other common symptoms. A significant group of infants may have relatively little in terms of specific neurological signs but may have nonspecific symptoms such as apnea, irritability, poor feeding, hypotonia, or vomiting.

The etiology of neonatal CCSVT remains unclear and a number of risk factors have been suggested. These include a range of maternal pregnancy complications, including preeclampsia and maternal diabetes; fetal/neonatal complications including meconium aspiration, dehydration, and sepsis; and underlying fetal conditions such as congenital heart disease and thrombophilias. However, none of the associations is particularly strong and it seems likely that CCSVT is the result of a multihit pathogenesis.

The presentations of childhood CCSVT can be subtle and varied. Seizures, loss of consciousness or altered consciousness, focal neurological deficits, headache, and symptoms of raised intracranial pressure have all been reported. Some children are in fact asymptomatic and CCSVT is discovered on central nervous system (CNS) imaging that was performed for other reasons.

The cause of CCSVT in many children remains unknown; however, many cases are associated with local infections/inflammation. Otitis media and mastoiditis can be associated with sigmoid and transverse sinus thrombosis. Severe dehydration or systemic illness (viral, bacterial or inflammatory) can be associated, despite no apparent direct link, to the cerebral circulation. CCSVT is not an uncommon site for thrombotic complications in children with leukemia, especially when treated with L-asparaginase.

In adults, the most frequent but least specific symptom is severe headache, either of subacute or acute onset, present in 90% of patients; about 40% have seizures. Routine non-contrast and contrast head CT scans and brain MRI scans often are unrevealing, resulting in missed diagnoses, unless CT venogram or MR venogram is requested specifically.

Approximately 40% of patients with CCSVT have a hemorrhagic infarct, which is a consequence of venous occlusion. LMWH, or alternatively UFH if neurosurgical decompression is anticipated, is recommended (AHA/ASA 2011, EFSN 2010) in acute CCSVT, even if some parenchymal hemorrhage is present. Currently, there is no available evidence from randomized controlled trials regarding the efficacy or safety of systemic or local thrombolytic therapy in CCSVT. In a minority of patients in whom large venous hemorrhagic infarcts result in brain displacement and herniation, decompressive surgery is the only life-saving option. The role for DOACs is not defined, although small case series have been published with good results. Particularly given the decreased risk of intracranial hemorrhage, these agents may be an attractive option. The optimal duration of anticoagulant therapy is unknown. After a first

episode of CSVT, expert guidelines recommend anticoagulation for: (i) 3 months if the thrombosis was associated with a transient risk factor, (ii) 6 to 12 months if the event was unexplained and no high-risk thrombophilia has been detected, and (iii) long term if a high-risk thrombophilia is detected or the event is recurrent. At the time of writing, a randomized controlled trial of short- vs long-term anticoagulation in adults is recruiting.

There is significant variation in treatment of neonatal CSVT, most likely related to uncertainty about the true risk of bleeding when neonates with CSVT are given anticoagulation therapy. The ACCP guidelines suggest anticoagulation for all affected neonates unless there is substantial intracerebral hemorrhage. Alternatively, the American Heart Association guidelines suggest monitoring with sequential imaging and anticoagulation only in the presence of thrombus progression. In general, anticoagulation is an accepted component of therapy for all childhood CSVT, but this must be managed around any early surgical interventions that are required. Many authors suggest the use of anticoagulation in the presence of hemorrhage unless it is severe; the amount of hemorrhage that should preclude anticoagulation is not well delineated and it is probably better to err on the side of caution. In neonates and children, initial UFH transitioning to LMWH is the most common therapy and durations are similar to adults. There is little evidence to support thrombolysis.

Renal vein thrombosis

In adults, the classical symptom triad of acute renal vein thrombosis (RVT)—namely, acute flank pain, hematuria, and sudden deterioration of renal function—is uncommon. More common is a chronic course with subtle worsening of renal function, progressive proteinuria, and edema, often without pain or hematuria. As many as 30% to 50% of patients with chronic nephrotic syndrome have evidence of RVT, and it is not uncommonly bilateral and often protrudes into the IVC. Nephrotic syndrome leads to hypercoagulability, which may be the result of urinary AT loss, free protein S deficiency secondary to an increase in C4b-BP, or unknown causes. Diagnosis is made by Doppler ultrasound or MR venography. Thrombolytic therapy should be considered in case of acute thrombosis, particularly if there is bilateral disease or impending renal failure. Anticoagulation therapy is indicated. The length of anticoagulant therapy depends upon whether the thrombotic event was associated with a transient prothrombotic risk factor or the patient has permanent risk factors or a higher risk of thrombophilia.

RVT in neonates is the most common type of spontaneous venous thrombosis. Infants of diabetic mothers

are at particular risk, but perinatal asphyxia and dehydration are also associated. Outside of the neonatal period, RVT is uncommon in children. The pathogenesis of this entity is not vascular access-related and studies indicate that the thrombotic process begins in the renal microvasculature and then extends out into the renal veins and potentially the IVC (in 50% to 60% of cases). This is important because it means the kidney damage, which is usually the cause of acute death from renal failure (3% in untreated patients) or the cause of long term renal impairment (75%) or hypertension (15%), is unlikely to be resolved by removal of the large vessel thrombosis within the IVC or renal veins, as would be achieved by thrombectomy. Approximately 25% of cases are bilateral, supporting the concept that this disease is related to something occurring within the renal parenchyma vasculature as distinct from large vessels. Recurrence rates are very low, and subsequent risk of other thrombosis does not appear to be increased. Anticoagulation is recommended in unilateral disease with or without extension into the IVC, and thrombolysis should be considered in bilateral disease with renal impairment. While the evidence quality is low, treatment appears to give reductions in mortality and long-term hypertension and is currently recommended.

Retinal vein thrombosis

Thrombosis can occur as central retinal vein occlusion (CRVO) or as branch retinal vein occlusion. CRVO has a prevalence of 1 in 250 to 1,000 in individuals over 40 years of age. The presence of classic arterial cardiovascular risk factors, such as hypertension, hyperlipidemia, and especially diabetes, has been associated with retinal vein occlusion. An association with inherited or acquired thrombophilia has not been convincingly demonstrated. Unfortunately, there is very little high-quality evidence on which to judge the utility of antiplatelet or anticoagulant therapy for CRVO. One small (67-patient) randomized trial indicates that 90 days of LMWH treatment may be more effective than aspirin for the prevention of visual loss in patients with retinal vein occlusion; however, the optimal duration of anticoagulant therapy is not known because long-term comparisons of anticoagulant vs antiplatelet vs no therapy have not been performed.

Arterial thromboembolism

General comments

The hematologist typically does not get called upon for the management of patients with ischemic disease that is due to arteriosclerosis. Therefore, this chapter does not discuss the pathophysiology of arteriosclerosis and its role in

arterial occlusive disease or the management approaches aimed at modifying an individual's arteriosclerosis risk factors, such as weight reduction, cessation of smoking, increased physical activity, and treatment of diabetes mellitus, hypertension, and hyperlipidemia. References to the major treatment guidelines are listed, for the interested reader, in the Bibliography. Considerations unique to a person who is young, has no significant arteriosclerosis risk factors, or has a personal or family history of thrombophilia is discussed below.

Arterial thrombosis in the absence of arteriosclerosis

Arterial thromboembolic events in the young person (<50 years of age) are rare, unless significant arteriosclerosis risk factors are present. No matter which territory the arterial thrombotic event occurs in, a number of risk factors and associated disorders should be investigated to clarify the etiology of the event (Table 9-2). As for specific arterial territories, in the case of upper-extremity arterial thromboembolism, thoracic outlet syndrome should be considered; in lower-extremity claudication or arterial thromboembolism, popliteal artery entrapment syndrome, cystic adventitial disease of the popliteal artery, fibromuscular dysplasia of the lower-extremity arteries, and endofibrosis of the iliac artery should be considered; and in the case of stroke, spontaneous or traumatic cervical artery dissection should be considered.

Relatively little is known about thrombophilias predisposing to arterial thrombosis. Arterial thrombosis is a classifying clinical criterion for APS. Whether young patients with otherwise unexplained arterial thromboembolic events and an inherited "strong" thrombophilia (such as protein C, protein S, or AT deficiency), would benefit from taking an anticoagulant (in addition to or instead of antiplatelet therapy) is not known. This, along with the lack of high-quality evidence that these inherited thrombophilias are linked to arterial thrombosis, leads many clinicians to avoid searching for inherited thrombophilia in patients with arterial events.

Pediatric considerations

Non-CNS arterial thrombosis in children within tertiary pediatric hospitals occurs with slightly less frequency than venous thrombosis. Arterial thrombosis in children is predominantly iatrogenic, related to vascular access (arterial puncture or catheter placement). Femoral artery thrombosis following cardiac catheter; peripheral artery thrombosis following arterial line placement, especially in neonates; and umbilical artery thrombosis (also in neonates) are the most common clinical situations encountered. Thrombosis in arteries of transplanted solid organs

is another significant clinical entity. Spontaneous arterial thrombosis (including the aorta) can occur but is rare. Coronary artery thromboses are almost always in the setting of giant aneurysms secondary to Kawasaki disease. Peripheral artery disease classically seen in vasculopathic adults is almost never seen in children.

The degree of tissue ischemia depends upon the degree of occlusion and the presence or absence of a collateral circulation. Immediate removal of the catheter may restore blood flow and relieve distal ischemia, especially as any coexistent arterial spasm resolves over subsequent minutes to hours. Anticoagulation, thrombolysis, and surgical thrombectomy are all reported as appropriate therapy depending on the degree of ischemia, and the requirement for future vascular access for therapeutic procedures (eg, cardiac catheters). Initial anticoagulation with heparinoid is often adequate therapy. Thrombolysis or surgical intervention may be required if organ or limb infarction is imminent. The optimal duration of anticoagulation therapy, and the role of subsequent platelet inhibition therapy, remain unknown. True rates of long-term consequences such as claudication or limb length discrepancy (due to growth failure) remain unknown.

Atrial fibrillation and stroke prevention

The hematologist is occasionally asked about the risk-benefit trade-offs associated with anticoagulation in a patient with nonvalvular atrial fibrillation. Detailed information relevant to this clinical decision can be found elsewhere (Table 9-3), but for most patients with AF, the risk of bleeding with anticoagulation using either a DOAC or a VKA is outweighed by the benefit. The rare exceptions are patients who are either at very low risk of stroke or at exceptionally high risk of anticoagulation-related major bleeding.

Neonatal stroke

Neonatal stroke, defined as a cerebrovascular event that occurs between 28 weeks gestation and 7 days of age, occurs in 1 in 250 live births. There is a male predominance. Approximately 60% present with early symptoms, mostly seizures and nonfocal neurological signs during the first 3 days of life. The seizures are often focal in nature. About 40% of affected children do not have specific symptoms in the neonatal period and are only recognized later with the emergence of motor impairment, developmental delay, specific cognitive deficiency, or seizures. It is often difficult to determine whether the stroke occurred in utero, at the time of delivery, or within the first week. Most neonatal stroke occurs in the distribution of the left-middle cerebral artery. MRI and angiography are the best tests to determine

Table 9-2 “Unexplained” arterial thromboembolism: suggested approach to structured evaluation

A. Is arteriosclerosis the underlying problem?

Arteriosclerotic changes demonstrated on imaging studies or pathology specimens?

Arteriosclerosis risk factors present?

Cigarette smoking

High blood pressure

High low-density lipoprotein (LDL) cholesterol

Low high-density lipoprotein (HDL) cholesterol

High lipoprotein(a)

Diabetes mellitus

Obesity

Family history of arterial problems in young relatives (<50 years of age)

B. Has the heart been thoroughly evaluated as an embolic source?

Atrial fibrillation—EKG, Holter, or event monitor

Patent foramen ovale—obtain cardiac echo: transthoracic echo with bubble study and Valsalva maneuver; if negative, consider transesophageal echo with bubble study

C. Other causes

Is the patient on estrogen therapy (contraceptive pill, ring, or patch; hormone replacement therapy)?

Does the patient use amphetamines, cocaine, or anabolic steroids?

Is there evidence for Buerger’s disease (does patient smoke tobacco or cannabis)?

Does patient have symptoms suggestive of a vasospastic disorder (Raynaud’s)?

Were anatomic abnormalities seen in artery leading to the ischemic area (web, fibromuscular dysplasia, dissection, vasculitis, external compression)?

Does patient have evidence of a rheumatologic or autoimmune disease (arthritis, purpura, or vasculitis)? Consider laboratory workup for vasculitis and immune disorder.

Is there a suggestion of an infectious arteritis?

Could the patient have hyperviscosity or cryoglobulins?

D. Thrombophilia workup for arterial events

Hemoglobin and platelet count (PVT and ET are also associated with increased arterial thrombotic events)

Antiphospholipid antibodies

Anticardiolipin IgG and IgM antibodies

Anti-β2-glycoprotein I IgG and IgM antibodies

Lupus anticoagulant

Flow cytometry to exclude PNH (if any evidence of hemolysis or cytopenias are present)

Homocysteine* (controversial, only if homocystinuria is suspected)

Lipoprotein(a) (in pediatrics)

Do *not* test for MTHFR polymorphisms, PAI-1 or tPA levels or polymorphisms, fibrinogen or factor VIII activities.

Suggest *not* to test for FVL mutation, prothrombin gene mutation, protein C/S activity and antithrombin activity is not established

EKG, electrocardiogram; ET, essential thrombocythemia; FVL, factor V Leiden; PAI-1, plasminogen activator inhibitor-1; PNH, paroxysmal nocturnal hemoglobinuria; PVT, portal vein thrombosis; tPA, tissue plasminogen activator.

* Uncertain clinical utility.

Table 9-3 Key resources for use of antithrombotic drugs in arteriosclerotic occlusive arterial disease, atrial fibrillation, and valvular heart disease

Disease/condition	Antithrombotic therapy guidelines	
	ACCP (<i>Chest</i> 2016)	AHA/ACC
Peripheral arterial disease	Alonso-Coello P et al.	Smith SC et al., 2011
TIA and stroke	Lansberg MG et al.	Furie KL et al., 2011
Coronary artery disease	Vandvik PO et al.	Smith SC et al., 2011
Myocardial infarction	Vandvik PO et al.	Wright RS et al., 2011
Atrial fibrillation	You J et al.	Fuster V et al., 2011
Valvular and other heart disease	Whitlock R et al.	Bonow RO et al., 2008

ACCP, American College of Chest Physicians; AHA/ACC, American Heart Association/American College of Cardiology; TIA, transient ischemic attack.

extent of disease. The mechanism of stroke in the different groups of newborns with stroke (term vs preterm; symptomatic neonates vs those with a delayed presentation, sick vs well) is likely to be different, and as yet risk factors remain poorly defined. At the time of diagnosis, though, it is important to determine whether the thrombotic event was related to an underlying disorder, such as congenital heart disease or so-called TORCH (toxoplasmosis, syphilis, herpes, cytomegalovirus) infections, which are passed in utero from the mother to the developing fetus; systemic bacterial infections, or metabolic diseases. Maternal drugs and medical conditions, placental disorders, perinatal asphyxia, and birth trauma all have been associated with neonatal cerebrovascular events. Recent studies have shown there is no association with inherited thrombophilia and therefore testing for this is of no benefit. Recurrence rates for most perinatal/neonatal arterial ischemic stroke are extremely low, and hence there is no justification for anticoagulant or antiplatelet therapy once the diagnosis is made. In cases of cardioembolic stroke (with proven embolic source remaining in the heart), or traumatic major vessel dissection, then anticoagulation or antiplatelet therapy is usually warranted. Neonatal supportive care remains the mainstay for all infants, including managing seizures, glucose and blood pressure, and preventing infection. Fifty percent of infants with perinatal events are neurologically normal by 12 to 18 months of age. Long-term sequelae, such as mild hemiparesis, speech or learning problems, behavioral problems, and seizures, are more likely to persist in patients who present outside

the newborn period. There is no specific evidence that early rehabilitation therapy improves long-term outcome, but it is a very reasonable extrapolation given the role of early intervention in improving the neurological outcome for many other infants who suffer neurological insults in early life. Physical, occupational, and speech therapy may all be required, as well as specific learning assistance in later life.

The recurrence risk for subsequent pregnancies appears to be low in most cases.

Childhood stroke

Stroke is the most common cause of brain attack (focal neurological deficit) symptoms in adults, accounting for approximately three-quarters of cases in patients presenting to the emergency department. In contrast, there is a much lower a priori probability of stroke in children presenting with brain attack symptoms. Migraine is the most common cause of sudden onset focal neurological symptoms and signs, first febrile or afebrile seizures the second most common diagnosis, and then Bell's palsy before ischemic or hemorrhagic stroke, and conversion disorders. Thus, less than 10% of children who present to an emergency department with acute focal neurological symptoms and signs have stroke. More common presenting features of stroke include hemiparesis (22% to 100%), headache (16% to 45%), altered mental state (12% to 24%), speech disturbance (28% to 55%), altered consciousness (24% to 52%), and seizures (11% to 58%). Age influences the clinical presentation, with seizures, altered mental state, and nonfocal signs being more likely in infants. History should include any evidence of recent head/neck injury or neck manipulation; varicella infection in the last 6 to 12 months; history or family history of migraine; and oral contraceptive pills or illicit drug use in adolescents. The nonspecific symptoms and alternative potential diagnoses often lead to delay in diagnosis of childhood stroke, with multiple studies reporting the average time from symptom onset to diagnosis as being in excess of 20 hours. This obviously has massive consequences in terms of the use of acute therapies such as thrombolysis or endovascular procedures.

Arteriopathies (vasculopathies) are the commonest cause of arterial ischemic stroke in children, accounting for about 50% of cases. Cardioembolic strokes frequently occur in children with underlying congenital heart disease and most often around the time of major surgical procedures. These may be in the anterior or posterior cerebral circulations and are usually single events, although occasionally showers of embolic lesions can be seen on neuro-

imaging. The risk of recurrence usually relates to the flow abnormalities within the heart, the presence or absence of further source clot, and the effectiveness of anticoagulation. Dissection of major vessels, including the extracranial carotid artery or the vertebral basilar system, is not uncommon after minor trauma or twisting forces. Often formal angiography is required to exclude or confirm the diagnosis. Most protocols for initial imaging of pediatric stroke patients include extension of the vascular imaging to include the neck vessels to consider this potential diagnosis.

The role of thrombophilias in the etiology of pediatric stroke remains controversial. While many studies report associations between stroke and heterozygous thrombophilic states in children, the methodology of most studies is less than ideal, and the evidence that links the blood results to recurrence or outcome, and hence impacts on potential therapy, is weak.

A significant proportion of childhood strokes are truly cryptogenic, occurring in otherwise well children without any precipitating factors. Multiple other associations have been suggested, including iron deficiency; however, many events remain unexplained. Fortunately, in these cases the recurrence risk appears to be lower, but it is difficult to be totally reassuring to patients and their families. Stroke is very common in children with underlying sickle cell disease (see relevant chapter).

Anticoagulation (LMWH, UFH, warfarin) or antiplatelet (predominantly aspirin) therapy is aimed at reducing the risk of recurrence and maximizing the recovery of the ischemic penumbra surrounding the infarcted area. The evidence supporting any specific approach is relatively low, and the risk of increasing secondary hemorrhage must always be considered. In general, arteriopathies are thought to require antiplatelet therapy, while cardioembolic and dissection-related strokes are thought to require formal anticoagulation. The question of whether at presentation, anticoagulation therapy should be commenced with conversion to antiplatelet therapy once cardioembolic causes or dissection have been excluded—or alternatively, whether antiplatelet therapy should be commenced with conversion to anticoagulation once cardioembolic causes or dissection have been proven—remains unanswered. There are clear geographic differences in approach. The optimal duration of these therapies is unclear, but anticoagulation is frequently used for 3 months, while antiplatelet therapy is often prescribed for 12 months poststroke.

As many as 65% of affected children develop lifelong disabilities, such as neurological defects and seizures, and the risk of a second stroke is 20%. Despite therapy, mortality rates as high as 10% have been reported.

Thrombophilias

The terms *thrombophilia* and *hypercoagulable state* refer to hereditary or acquired predispositions to develop thrombosis. Although the clinical relevance of testing for these conditions has diminished somewhat in recent years, the hematologist must be familiar with the nature, limitations, and interpretation of such testing. It is important to note that first-degree relatives of patients who have experienced VTE (provoked less so than unprovoked) are at an increased risk of venous thrombosis, irrespective of thrombophilia test results. When assessing risk, selective testing in families with a strong history of VTE and, consequently, cosegregation of known and unknown genes in the early days of thrombophilia research, has resulted in an apparent stronger relative risk increase than more contemporary studies have established. This is particularly true for AT, protein C, and protein S deficiencies. Table 9-4 lists the prevalence and association with various clinical manifestations. Table 9-5 lists the risk of a first VTE in asymptomatic first-degree relatives of patients with VTE.

In the next section, we first discuss all inherited and acquired thrombophilias and end with a section dedicated to neonates and children.

Inherited thrombophilias

Family history of VTE

Simply having a family history of VTE is a risk factor for first-time VTE, no matter whether or not a known thrombophilia is detectable in the family. This additional risk is due to unknown or unmeasured risk factors. Having a first-degree relative with a history of VTE increases an individual's risk of VTE 2- to 4-fold. Young age of incident VTE, and/or an unprovoked clot in the affected relative, and having more than 1 affected first-degree relative all increase the likelihood of developing a first VTE. Whether a strong family history of VTE is a risk factor for recurrent VTE, and thus should be used in decision making on length of anticoagulation therapy after a first episode of VTE, is not known.

Factor V Leiden

General information

APC is a potent inhibitor of the coagulation system, cleaving the activated forms of factors V and VIII (FVa and FVIIIa) (Figure 9-3A and B, FVIIIa not shown). The FVL mutation, one of the most commonly identified inherited thrombophilias in populations of European ancestry, is a point mutation (G1691A) in the factor V gene, leading to a

Table 9-4 Prevalence of thrombophilia and relative risk estimates for various clinical manifestations

	Antithrombin deficiency	Protein C deficiency	Protein S deficiency	Factor V Leiden mutation (heterozygote)	Prothrombin 20210A mutation (heterozygote)
Prevalence in the general population	0.02%	0.2%	0.03%–0.13%	3%–7%	0.7%–4%
Relative risk for a <i>first</i> venous thrombosis	5–10	4–6.5	1–10	3–5	2–3
Relative risk for <i>recurrent</i> venous thrombosis	1.9–2.6	1.4–1.8	1.0–1.4	1.4	1.4
Relative risk for arterial thrombosis	No association	No consistent association	No consistent association	1.3	0.9
Relative risk for pregnancy complications	1.3–3.6	1.3–3.6	1.3–3.6	1.0–2.6	0.9–1.3

Table 9-5 Estimated incidence of a first episode of VTE in carriers of various thrombophilias (data apply to individuals who have at least 1 symptomatic, first-degree relative)

	Antithrombin, protein C, or protein S deficiency	Factor V Leiden, heterozygous	Prothrombin 20210A mutation	Factor V Leiden, homozygous
Overall (%/year, 95% CI)	1.5 (0.7–2.8)	0.5 (0.1–1.3)	0.4 (0.1–1.1)	1.8 (0.1–4.0)★
Surgery, trauma, or immobilization (%/episode, 95% CI)†	8.1 (4.5–13.2)	1.8 (0.7–4.0)	1.6 (0.5–3.8)	—
Pregnancy (%/pregnancy, 95% CI) (includes postpartum)	4.1 (1.7–8.3)	2.1 (0.7–4.9)	2.3 (0.8–5.3)	16.3‡
During pregnancy, %, 95% CI	1.2 (0.3–4.2)	0.4 (0.1–2.4)	0.5 (0.1–2.6)	7.0‡
Postpartum period, %, 95% CI	3.0 (1.3–6.7)	1.7 (0.7–4.3)	1.9 (0.7–4.7)	9.3‡
Oral contraceptive use (%/year of use, 95% CI)	4.3 (1.4–9.7)	0.5 (0.1–1.4)	0.2 (0.0–0.9)	—

★Based on pooled OR of 18 (8–40) and an incidence of 0.1% in noncarriers.

†These risk estimates mostly reflect the situation before thrombosis prophylaxis was routinely used.

‡Data from family studies; risk estimates lower in other settings.

VTE, venous thromboembolism.

factor V molecule with an arginine-to-glutamine substitution at position 506 (Arg506Gln, R506Q). This abolishes a cleavage site for APC and makes factor Va less susceptible to inactivation (Figure 9-3C). Based on the initial observation that APC did not appropriately prolong aPTT in a dose-dependent fashion, this defect was termed *activated protein C resistance* (APC resistance). FVL accounts for >90% of APC resistance. Other causes of APC resistance include less common genetic mutations of factor V (factor V Cambridge, factor V Liverpool) and acquired causes of APC resistance, including APLAs, pregnancy, and cancer. FVL is inherited in an autosomal-dominant fashion. The high prevalence of FVL in the general population suggests that it has led to evolutionary advantages, perhaps includ-

ing protection against massive postpartum hemorrhage, increased fecundity, and increased male sperm count.

Prevalence

The prevalence of heterozygous FVL is 3% to 8% in Caucasian populations and 1.2% in African Americans. It rarely is found in native African and Asian populations. Homozygous FVL occurs in 1 in 500 to 1,600 Caucasians.

Laboratory aspects

The diagnosis of FVL is made by genetic testing (ie, polymerase chain reaction [PCR]; some laboratories screen for FVL with an APC resistance assay). The currently used second-generation APC resistance assays, which are aPTT-

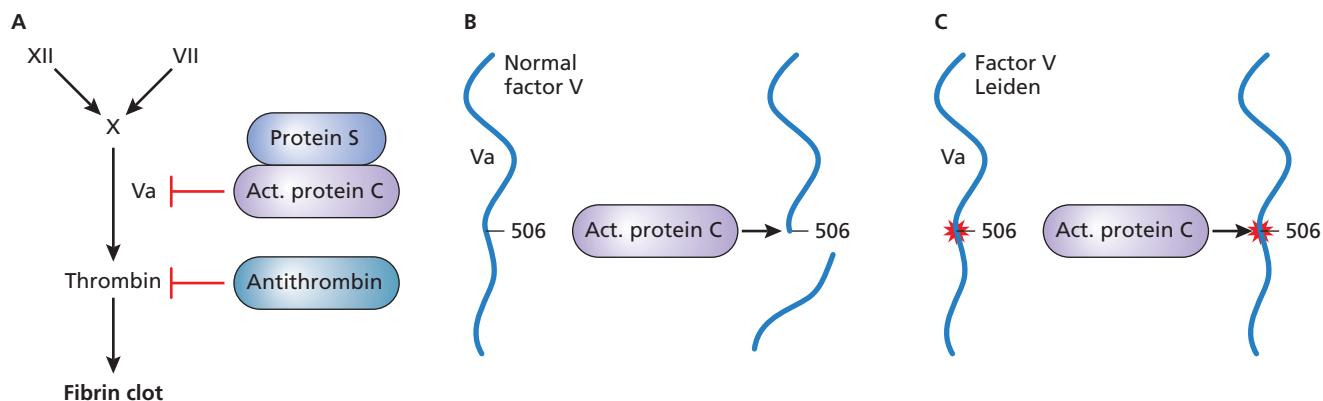


Figure 9-3 Simplified coagulation system with (A) sites of action of the natural anticoagulants; (B) method of inactivation of factor V; (C) demonstration of the inability of activated protein C to inactivate factor Va when the factor V Leiden mutation is present.

based coagulation assays using factor V–deficient plasma, are very sensitive and relatively specific for detection of the FVL mutation. An abnormal APC resistance test result, however, may be due to causes other than FVL, and therefore should be followed by the genetic FVL test.

Risk for thrombosis

Heterozygosity for FVL is mildly thrombophilic, leading to a 3- to 5-fold increased risk of first-time VTE. Homozygosity confers an 18-fold increased risk compared with individuals without the FVL mutation. Additional VTE risk factors—such as age, smoking, obesity, and particularly use of estrogens and pregnancy—increase the risk further. Among FVL carriers with a first-degree relative with VTE, the incidence of first VTE increased from 0.25% (95% confidence interval [95% CI], 0.12% to 0.49%) in the 15- to 30-year-old age group to 1.1% (95% CI, 0.24% to 3.33%) in persons older than 60 years of age. Half of the episodes of VTE were unprovoked, 20% were related to surgery, and 30% were associated with pregnancy or use of oral contraceptives.

The risk for recurrent VTE in FVL-heterozygous carriers is only modestly increased (odds ratio [OR] 1.56; 95% CI, 1.14 to 2.12) compared to individuals with a history of VTE without FVL. The risk of recurrence in individuals with homozygous FVL compared with those without FVL was estimated to increase 2.65-fold (95% CI, 1.2 to 6.0), although there are wide ranges in risk estimates. For practical purposes, there is no clinically meaningful association between FVL and arterial thromboembolic events in adults: a meta-analysis demonstrated the risk to increase 1.21-fold (95% CI, 0.99 to 1.49) in FVL carriers compared with noncarriers.

Management

Because heterozygosity for FVL confers only a mildly increased risk of VTE recurrence compared to individu-

als without FVL, its finding alone typically does not alter treatment decisions on duration of anticoagulation. Furthermore, asymptomatic family members of persons with FVL heterozygosity generally need not be tested. The possible exception is young women who may consider avoiding some oral contraceptives in case they are heterozygous for the FVL mutation; furthermore, the risk for pregnancy-related VTE may justify the use of postpartum (and in some cases, antepartum) prophylaxis with LMWH. As with other inherited thrombophilias, a patient with a strong family history who is contemplating discontinuation of anticoagulant therapy may wish to undergo FVL testing, since homozygosity for the mutation may significantly alter the estimated future risk of recurrence.

Prothrombin 20210 mutation

General information

A point mutation in the factor II gene in the noncoding region in nucleotide position 20210 (G20210A) is the second most commonly known inherited risk factor for venous thrombosis. Individuals who are heterozygous for this polymorphism have slightly higher levels of circulating prothrombin. It is inherited in an autosomal dominant fashion.

Prevalence

The mutation is found most commonly in individuals of southern European ancestry, with a prevalence throughout Europe of 0.7% to 4%. In the United States, it occurs in 2% of the general population and in 0.5% of the African American population. The prothrombin 20210 mutation is rare in other non-Caucasian populations. Homozygosity for the prothrombin 20210 mutations occurs, by calculation, in approximately 1 in 4,000 individuals of Caucasian heritage.

Laboratory aspects

Testing is done using genetic testing (PCR). Although the mutation leads to higher circulating factor II levels, it is not helpful in individual patients to use factor II activity or antigen levels as screening tests, because there is a wide overlap of levels between people with and without the mutation.

Risk for thrombosis

Heterozygosity for the prothrombin 20210 mutation is mildly thrombophilic, conferring a 3-fold increased risk of first-time VTE compared with noncarrier status. The effect of this mutation on the risk for first and recurrent VTE is very similar to FVL.

The risk for recurrent VTE in carriers of the prothrombin 20210 mutation compared with the absence of the mutation is, at most, modestly increased (OR 1.45; 95% CI, 0.96 to 2.2). Thus, treatment decisions on length of anticoagulant therapy are not based on the presence or absence of the heterozygous prothrombin 20210 mutation. Population-based data regarding the risk of thrombosis for homozygotes for the prothrombin gene mutation are not available. A summary of 70 cases of homozygous individuals published in the medical literature indicates a marked phenotypic heterogeneity. Data on the risk of recurrence of VTE in individuals with homozygous prothrombin 20210 mutation do not exist. Although some studies suggest a relationship between the prothrombin 20210 mutation and stroke and myocardial infarction risk in younger patients, meta-analysis has not demonstrated any clinically meaningful association between the prothrombin mutation and arterial thromboembolism.

Management

Because heterozygosity for the prothrombin 20210 mutation does not confer a statistically significant or clinically relevant increased risk of VTE recurrence, this finding does not alter length of anticoagulation treatment decisions. Furthermore, similar to the discussion about FVL, first-degree relatives of people who are heterozygous need not be tested routinely because they are known to be at increased risk if their relative has experienced symptomatic VTE. Again, the exception may be young women in the reproductive phase of their lives (Tables 9-5 and 9-6).

Protein C deficiency

General information

Protein C is a vitamin K-dependent protein, converted during the coagulation process to APC. APC acts as a natural anticoagulant. In complex with the cofactor protein S, it inactivates coagulation factors Va and VIIa, making them unavailable as cofactors during the coagulation

process (Figure 9-3A). Inherited protein C deficiency as a cause of thromboembolism was first described in 1981. Two types of deficiency are known, but their distinction is not clinically important with regard to the thrombotic risk they confer. Type I deficiency is defined as a quantitative deficiency with low functional protein C (activity) and immunologic (antigen) level; type II is defined as a qualitative deficiency with low activity but normal antigen level. Approximately 85% of the reported cases have type I deficiency, whereas 15% have type II deficiency. More than 160 mutations causing protein C deficiency have been described. It is inherited in an autosomal dominant fashion.

Prevalence

The prevalence of inherited protein C deficiency in the general population is approximately 1 in 500 to 600. By calculation, homozygous or double heterozygous protein C deficiency occurs in approximately 1 in 1 million individuals.

Laboratory aspects

When evaluating an individual for protein C deficiency, a protein C functional (activity) test should be performed, because obtaining only an antigen level misses type II deficiencies. Outside of research studies, there is no need to obtain protein C antigen levels. Because laboratory reports may report results only as "protein C normal," leaving it unclear whether an activity or antigen test was done, to avoid missing a type II deficiency, a physician may wish to clarify which test was actually performed. Falsely low protein C activity values may be seen with high levels of factor VIII and with lupus anticoagulants. The most common reason for low protein C levels is treatment with VKAs (Tables 9-1 and 9-2). Patients should stop VKAs before protein C activity testing is performed. Given the short half-life of 8 to 10 hours, 1 week should be long enough to ensure protein C levels have returned to normal. It is not known how many patients who carry a diagnosis of protein C deficiency truly have a congenital deficiency and how many have an erroneous diagnosis of protein C deficiency due to testing at an inappropriate time (eg, while on VKAs). Thus, the hematologist should always question the diagnosis until review of records and laboratory results has clarified that the timing of testing was correct and no confounding issues led to a transient decrease in protein C. A normal PT at the time of protein C testing is important to exclude vitamin K deficiency as a cause of decreased protein C activity. Repeat confirmatory testing of a low protein C level at a separate time point is also necessary. Confirmation of a hereditary defect by testing a parent or other relative is recommended.

Table 9-6 Estimated number of asymptomatic thrombophilic women or women with a positive family history for VTE who would have to avoid using oral contraceptives to prevent 1 VTE, and estimated number needed to test

Thrombophilia	Risk on OC per year, %	Risk difference per 100 women	N not taking OC to prevent 1 VTE	N of female relatives to be tested
Antithrombin, protein C, or protein S deficiency				
Deficient relatives	4.3*	3.6	28	56
Nondeficient relatives	0.7*			
Factor V Leiden or prothrombin 20210A mutation				
Relatives with the mutation	0.5*	0.3	333	666
Relatives without the mutation	0.2*			
Family history of VTE				
General population, no family history	0.04†	0.03	3333	none
General population, positive family history	0.08†	0.06	1667	none

Based on family studies as outlined in Table 9-5.

*Based on a population baseline risk of VTE in young women of 0.01% per year, a relative risk of VTE by use oral contraceptives of 4, and a relative risk of 2 of VTE attributable to positive family history.

OC, oral contraceptives;VTE, venous thromboembolism.

Risk for thrombosis

Protein C deficiency is considered to be one of the higher-risk thrombophilias. It is a risk factor mainly for VTE (Table 9-4). Rates of thrombosis vary widely among individuals and families with protein C deficiency. For asymptomatic relatives of probands with protein C deficiency and a first VTE, most studies suggest the risk of first VTE is increased between 4- and 7-fold (Table 9-5). The annual incidence of a first VTE is 1.5% in protein C-deficient individuals.

Protein C deficiency is only modestly associated with a risk of recurrent VTE (Table 9-4). In adults, a link between protein C deficiency and risk of arterial thrombosis has not been firmly established.

Management

Patients with protein C deficiency initiated on VKAs are at risk for warfarin-induced skin necrosis. This transient hypercoagulable state is related to abrupt declines in protein C activity (which was low to begin with) after the initiation of VKA. Any patient with acute VTE who is initiated on VKAs needs concurrent anticoagulation with a parenteral anticoagulant for at least 5 days and until the INR is >2.0, but this is particularly important in the person with known protein C or S deficiency. This concern is not relevant with the use of DOACs. With regard to the need for testing, similar considerations apply as for FVL. Given the somewhat higher risk increase associated

with protein C deficiency, particularly in families with a strong tendency to develop VTE, the presence of protein C deficiency may shift the decision toward extended duration of anticoagulation.

Protein S deficiency

General information

Protein S is also a vitamin K-dependent protein. Forty percent of protein S exists in a free form, and the remaining 60% in a complex with the transport protein called *C4b-binding protein* (C4b-BP). It is mostly free protein S that functions as a natural anticoagulant, by being a cofactor for APC to inactivate FVa and FVIIIa (Figure 9-3A).

Protein S deficiency was first described in 1984. More than 131 different mutations have been identified leading to protein S deficiency, which is an autosomal dominant disorder. Severe protein S deficiency due to homozygous or double heterozygous mutations can lead to early onset of VTE or severe neonatal purpura fulminans and death.

Protein S deficiency is classified into type I, a quantitative deficiency, in which both free and total protein S antigen levels are decreased; type II, a qualitative defect due to a dysfunctional protein, in which protein S activity is low, but free and total antigen levels are normal; and type III, a quantitative deficiency, in which free protein S antigen level is low and the total antigen level is normal. Type III deficiency is either due to a high C4b-BP plasma

concentration or to an abnormal binding of protein S to this carrier protein. The basis for type III deficiencies is not known, but it appears to encompass genetic and environmental factors. The majority of the known mutations (approximately 93%) lead to quantitative deficiencies (ie, type I and III). Protein S deficiency is inherited in an autosomal dominant fashion. Confirmation of a hereditary defect by testing a parent or other relative is recommended.

Prevalence

Reported prevalence in the general population varies between 1 in 800 and 1 in 3,000, but due to difficulties in establishing the normal range of protein S concentrations and in making an accurate diagnosis, the true prevalence of protein S deficiency is not known (Table 9-4).

Laboratory aspects

Measuring either free protein S antigen or protein S activity detects most cases of protein S deficiency. However, because these individual tests, if done in isolation, can occasionally yield falsely normal results, it is advisable to include both functional testing (protein S activity) and immunologic testing (free protein S antigen) if the clinical suspicion for protein S deficiency is high. High factor VIII levels, the presence of the FVL mutation, or the presence of a lupus anticoagulant may give falsely *low* protein S activity values.

Protein S levels are low in the setting of estrogen therapy, pregnancy and postpartum period, liver disease, nephrotic syndrome, disseminated intravascular coagulation, and therapy with VKAs (Tables 9-7 and 9-8). Congenital protein S deficiency cannot be diagnosed in these circumstances. A patient needs to have been off VKA for 3 weeks before protein S levels can be considered reliable, as its half-life is long (40 to 60 hours). Thus, as with protein C deficiency, timing of the testing is essential to making a correct diagnosis and repeat confirmatory testing (including both free antigen and activity) on a new plasma sample is advisable. A normal PT at the time the sample is obtained excludes vitamin K deficiency as a cause for abnormal protein S activity or antigen levels. Critical scrutiny as to whether a patient said to have protein S deficiency truly has the disorder is appropriate.

Risk for thrombosis

Protein S deficiency has traditionally been considered to be one of the higher-risk thrombophilias. Because of the genetic diversity of mutations associated with protein S deficiency, rates of thrombosis vary widely among individuals and families with known defects. Although there

Table 9-7 Conditions associated with acquired coagulation factor deficiencies

Factor	Conditions associated with decreased factor levels
Protein C	Acute thrombosis
	VKA therapy
	Vitamin K deficiency
	Liver disease
	Protein-losing enteropathy
Protein S	Acute thrombosis
	VKA therapy
	Vitamin K deficiency
	Liver disease
	Inflammatory states
Antithrombin	Estrogens (contraceptives, pregnancy, postpartum state, hormone replacement therapy)
	Protein-losing enteropathy
	Acute thrombosis
	Heparin therapy
	Liver disease
	Nephrotic syndrome
	Protein-losing enteropathy
	DIC
	Sepsis
	Asparaginase chemotherapy

DIC, disseminated intravascular coagulation;VKA, vitamin K antagonist.

is considerable variability among reports, most family cohort studies have found a relatively weak association between protein S deficiency and VTE risk (Table 9-4). Interestingly, protein S deficiency seems to have no association with increased VTE risk in some population-based case-control studies. The annual incidence of first VTE is 1.9% in protein S-deficient individuals from families with thrombosis.

Protein S deficiency is only modestly associated with a risk of recurrent VTE (Table 9-4). A link between protein S deficiency and increased risk for arterial thrombosis has not been well established. The heterogeneity in the clinical phenotype of patients with protein S deficiency must be taken into consideration when making decisions on anticoagulant treatment and family counseling.

Management

The implications of finding inherited protein S deficiency in an individual are similar to those discussed for the person found to have protein C deficiency. Diligent overlap

Table 9-8 Influence of acute thrombosis, heparin, vitamin K antagonists, and DOACs* on thrombophilia test results

Test	Acute thrombosis	Unfractionated heparin (UFH)	Low-molecular-weight heparin (LMWH)	Vitamin K antagonists (VKA)	Direct oral anticoagulant (DOAC)*
Factor V Leiden genetic test	Reliable	Reliable	Reliable	Reliable	Reliable
APC [†] resistance assay	Reliable [‡]	? [‡]	? [§]	Reliable [‡]	Likely not reliable [§]
Prothrombin 20210 genetic test	Reliable	Reliable	Reliable	Reliable	Reliable
Protein C activity	?	Reliable	Reliable	Low	? [§]
Protein S activity	May be low	Reliable	Reliable	Low	? [§]
Antithrombin activity	May be low	May be low	May be low	Occasionally elevated ^{**}	Probably reliable if chromogenic assay is used
Lupus anticoagulant	Reliable [¶]	? [#]	? [#]	May be false positive	False positive likely
Anticardiolipin antibodies	Reliable [¶]	Reliable	Reliable	Reliable	Reliable
Anti-β ₂ -glycoprotein I antibodies	Reliable [¶]	Reliable	Reliable	Reliable	

*Dabigatran, rivaroxaban, apixaban, edoxaban.

[†]APC, activated protein C.

[‡]Reliable if the assay is performed with factor V-depleted plasma; thus, clinician needs to inquire how the individual laboratory performs the assay.

[§]Depending on the way the assay is performed results may be unreliable; health care provider needs to contact the laboratory and ask how the specific test performs in the presence of the drug in question.

^{||}Probably reliable, but limited data in literature.

[¶]Test often positive or elevated at time of acute thrombosis, but subsequently negative.

[#]While many test kits used for lupus anticoagulant testing contain a heparin neutralizer making these tests reliable on UFH and possibly LMWH, clinicians need to inquire with their laboratory how their individual test kit performs in samples with UFH and LMWH.

^{**}A few case reports show that VKA can lead to an increase in antithrombin levels in selected families.

of parenteral anticoagulants upon initiation of VKAs for at least 5 days and until the INR is >2.0 is important to avoid warfarin-induced skin necrosis. As with protein C deficiency, this is not a concern with DOACs. Individuals with a first unprovoked episode of VTE who have a strong family history of VTE (and are contemplating discontinuation of anticoagulants) may wish to undergo protein S testing because a decrease in protein S activity may shift the decision toward extended duration of anti-coagulation.

Antithrombin deficiency

General information

AT is an enzyme that interrupts the coagulation process mostly by inhibiting thrombin (Figure 9-3A), activated factor X (factor Xa), and activated factor IX (factor IXa). It used to be referred to as antithrombin III (ATIII). AT deficiency was first described in 1965. Quantitative (type I) and qualitative (type II) defects exist. Type II deficiencies consist of defects affecting: (i) the thrombin-binding region, (ii) the heparin-binding region, and (iii) a variety of other AT molecule regions. More than 130 different genetic mutations are known. AT deficiency is inherited in an autosomal dominant fashion.

Prevalence

Inherited AT deficiency occurs in 1 in 500 to 5,000 people. Deficiencies are typically heterozygous, as homozygous deficiencies are almost always incompatible with life. In the general population, type II deficiencies are the more prevalent subtype, accounting for 88% of all AT deficiencies. A majority of these type II deficiencies are heparin-binding defects, which are not very thrombogenic. Causes of acquired AT deficiency can be found in Table 9-7.

Laboratory aspects

Testing for AT deficiency should be performed using a functional assay to detect both quantitative and qualitative defects. Heparin therapy can decrease AT levels by 30% (Table 9-8). Testing is best performed a few weeks after the initial thrombotic event and may best be done when a patient is not on heparin. No one should be diagnosed as having AT deficiency on the basis of 1 single abnormal test result, and a familial deficiency should be confirmed in a relative. An abnormal result should lead to repeat testing on a new blood sample. Because type II AT deficiency due to a heparin-binding defect appears to be much less thrombogenic than type I and other type II subtypes, differentiation of the AT deficiency subtype may be important for

clinical purposes. Specialized AT assays (AT activity in the absence of heparin) or gene sequencing need to be used for that purpose, but they are not widely available.

Risk for thrombosis

AT deficiency overall is considered to be one of the higher-risk thrombophilias. Type I and type II mutations affecting the thrombin-binding domain can be associated with VTE in nearly 50% of affected family members, although this may also be the result of selective testing of thrombophilic families. The prevalence of VTE in individuals with a defect in the heparin-binding site is much lower; only 6% of such individuals develop a VTE.

Once anticoagulation is stopped, the risk of recurrent VTE in individuals with AT deficiency is considered to be high, although later studies found a much weaker association with recurrent VTE. A large family study showed no association between AT deficiency and arterial thromboembolism.

Management

Long-term anticoagulation is usually recommended for patients with AT deficiency who have had a symptomatic VTE, although this may be inappropriate in patients with a provoked VTE and/or absence of a strong family history for VTE. Asymptomatic individuals with AT deficiency typically should receive VTE anticoagulant prophylaxis in high-risk situations. AT concentrate is available, either derived from the plasma of human donors or transgenically produced in goat milk. Because of a lack of high-quality evidence, the role of AT concentrate in clinical practice is not yet established.

Acquired thrombophilias

Cancer

General information

Approximately 20% of all VTEs occur in patients with cancer. About 5% of patients with unprovoked VTE have a previously undiagnosed cancer at the time of the VTE, and another 10% of patients with unprovoked VTE will be diagnosed with a cancer in the year following the VTE diagnosis. Evaluation for occult cancer should be considered in selected patients, such as those with recent weight loss and other unexplained symptoms or abnormalities on routine laboratory testing, such as anemia. Patients presenting with unprovoked VTE who are not up-to-date on age- and gender-appropriate cancer screening (eg, colorectal cancer screening, mammography, pap testing) should be encouraged to become so. Recent studies show that extensive screening (eg, computed tomography of the chest/

abdomen/pelvis, or PET/CT) for cancer in all patients with unprovoked VTE does not result in decreased cancer-associated morbidity or improved survival. Similar to adults, children with cancer are at increased risk for the development of VTE, but the majority of these VTEs are related to central venous catheters or cancer therapy, such as asparaginase or high-dose corticosteroids.

Management

Based on superior efficacy in several randomized comparisons with warfarin, LMWH is the standard of care for cancer-associated VTE. Guidelines from both the ACCP and the National Cancer Center Network recommend that patients with cancer-associated VTE receive LMWH monotherapy for at least the first 6 months after diagnosis. The initial total daily dose of LMWH mimics that used for treatment of noncancer acute VTE; after 1 month, the daily dose can probably be reduced by 20% to 25%. Randomized studies that compared edoxaban or rivaroxaban to LMWH for the treatment of cancer-associated thrombosis showed the DOACs to be noninferior to LMWH for the composite endpoint of recurrent VTE and major bleeding. In both studies, there appeared to be a relative increase in gastrointestinal bleeding with the DOAC. Overall, DOACs appear to be a reasonable alternative to LMWH for the treatment of CAT, and the choice for an individual patient should be made considering potential drug interactions, organ function, risk of bleeding, cost, and patient preference.

Myeloproliferative disorders

General information

Essential thrombocythemia (ET) and polycythemia vera are associated with a substantial risk for thrombosis (arterial more commonly than venous). A gain-of-function mutation of the Janus kinase-2 (JAK2) enzyme, the *JAK2* V617F mutation, is found in nearly 100% of patients with polycythemia vera and in 50% of those with ET. Some studies show that the presence of the *JAK2* V617F mutation is associated with an increased risk of thrombosis, either arterial or venous, in patients with ET, and may be somewhat dependent on variant allele frequency. At present, however, there are no data to suggest that therapeutic anticoagulation decisions should be based on the presence or absence of the mutation. These disorders are discussed in more detail in Chapter 16.

Splanchnic vein thrombosis and *JAK2* V617F mutation

The *JAK2* V617F mutation commonly is found in patients with splanchnic vein thrombosis (Budd-Chiari syndrome and portal, mesenteric, and splenic vein thrombosis),

occurring in approximately a third of such patients. Only about half of these *JAK2V617F*-mutation-positive patients have an MPN at the time of the diagnosis of their thrombotic event. *JAK2V617F*-mutation-positive patients with splanchnic vein thrombosis are more likely to develop an MPN during follow-up than patients with splanchnic vein thrombosis without the mutation. Thus, patients with splanchnic vein thrombosis who are found to have the *JAK2 V617F* mutation should be followed very closely to facilitate early detection of the development of clinical signs of an MPN. One can similarly argue that the *JAK2V617F*-mutation-negative patients should be followed just as closely, because up to 10% of these patients also develop an MPN.

Other VTEs and *JAK2 V617F* mutation

In patients with nonsplanchnic vein thrombosis, the prevalence of the *JAK2 V617F* mutation was found to be around 2% in a Dutch case-control study. The presence of the *JAK2V617F* mutation without symptoms of an MPN is not significantly associated with increased risk of first VTE (OR 4.5; 95% CI, 0.5 to 40.9). More importantly, none of the carriers had progression to an MPN over a 6-year follow-up period. This argues against screening patients with nonsplanchnic vein thrombosis for the *JAK2 V617F* mutation.

Paroxysmal nocturnal hemoglobinuria

General information

PNH is a clonal hematopoietic stem cell disorder resulting from an acquired mutation of the phosphatidylinositol-glycan class A gene, leading to absent or decreased cell surface expression of glycoprotein (GP) I–anchored proteins on the surface of blood cells. PNH is associated with increased risk of venous and arterial thrombosis, which most often occurs in intra-abdominal veins, particularly the hepatic veins (Budd-Chiari syndrome). Cerebral and peripheral vein thromboses also occur, but less commonly. The pathophysiology of thrombosis is not well understood, and no consistent abnormalities have been found.

Management

Screening for PNH by peripheral blood flow cytometry for CD55 and CD59 is warranted in thrombophilia evaluations of patients with venous or arterial thrombosis plus unexplained hemolysis or peripheral blood cytopenias. Antithrombotic therapy can be used for the treatment and secondary prevention of PNH-associated thrombosis, but “breakthrough” clotting events are well described. Long-term treatment with the complement inhibitor eculizumab appears to reduce the risk of thromboembolism (and improve life expectancy) in patients with PNH.

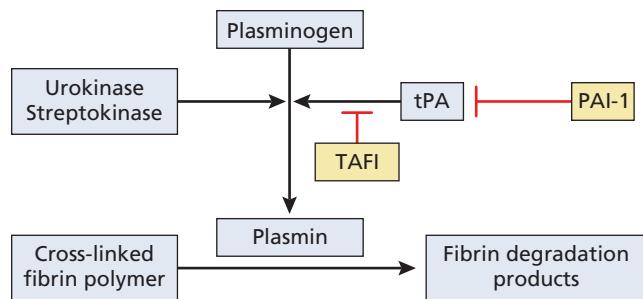


Figure 9-4 Fibrinolysis. TAFI, thrombin-activatable fibrinolysis inhibitor; tPA, tissue plasminogen activator.

Abnormalities in fibrinolysis

A variety of parameters of fibrinolysis (Figure 9-4) have been investigated as potential causes of thrombophilia. Investigation of these parameters has been challenging because coagulation assays do not reliably reflect fibrinolysis of formed thrombi. Studies often have yielded conflicting or inconclusive results regarding an association of antigen levels, enzyme activity, or certain polymorphisms and the risk for arterial or venous thrombosis. Given the variability of data associating impaired fibrinolysis to arterial and venous thrombosis and the imprecision of available assays, workup for abnormalities in the fibrinolytic pathway (ie, testing for plasminogen, tPA, plasminogen activator inhibitor-1 [PAI-1], and thrombin-activatable fibrinolysis inhibitor [TAFI]), with the knowledge we have at present, is not useful. Results do not explain the etiology of a thrombotic event in an individual patient, and they do not influence decision making regarding length of anticoagulant therapy.

Hormonal therapy and pregnancy

The increased VTE risk associated with hormonal contraceptives and pregnancy is discussed in Chapter 3.

Antiphospholipid antibodies

General information

APLAs are acquired autoantibodies directed against phospholipids and phospholipid-binding proteins, such as β_2 -glycoprotein I and prothrombin. They are associated with arterial thromboembolism, VTE, and pregnancy complications. A variety of different mechanisms leading to thrombosis have been proposed, but the precise pathophysiologic explanation for the clinical phenomena is not known. Diagnosis of APS requires objectively documented venous or arterial thrombosis, unexplained recurrent (3 or more) early (<10 weeks of gestation) miscarriages or 1 or more late pregnancy losses, or pregnancy complications associated with placental insufficiency together with persistent

laboratory evidence of APLAs, tested at least 12 weeks apart. The syndrome can occur either as primary APS (not associated with any other diseases) or secondary APS (associated with autoimmune diseases, malignancy, or drugs). Importantly, based on the definition of the syndrome, the clinical variation in phenotype is large, with some patients experiencing all manifestations of APS, whereas the same diagnosis is made in patients with, for example, a provoked VTE and 2 consecutive positive test results. From this it follows that the prognosis and inferences about prognosis cannot be easily generalized to all patients who have a diagnosis of APS.

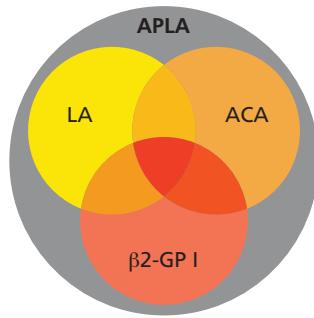
Prevalence

The prevalence of APS is poorly defined, but APLAs are found in nearly 50% of patients with systemic lupus erythematosus and up to 5% of the general population. Nearly 40% of patients with systemic lupus erythematosus meet diagnostic criteria for APS.

Testing

Laboratory evidence of an APLA is defined as: (i) moderately or highly positive (>40 GPL or MPL, or above the 99th percentile of a laboratory's own reference population) immunoglobulin G (IgG) or immunoglobulin M (IgM) anti- β_2 -glycoprotein I antibodies; or (ii) moderately or highly positive IgG and IgM anticardiolipin antibodies; or (iii) evidence of a lupus anticoagulant (sometimes called a lupus inhibitor) (Figure 9-5). Lupus anticoagulants are detected when phospholipid-dependent clotting times (eg, aPTT, Russell viper venom time) are prolonged. False-positive lupus anticoagulant test results are not uncommon, occurring frequently in patients who are on oral anticoagulants (including the newer direct thrombin and Xa inhibitors). False-negative results may occur if the blood sample was suboptimally centrifuged and the

Figure 9-5 Antiphospholipid antibodies (APLAs) with their different subtypes. ACA, anticardiolipin antibody; β_2 -GPI, anti- β_2 -glycoprotein I antibodies; LA, lupus anticoagulant. The figure shows the various potential patterns in the presence of types of APLAs amongst patients.



prepared plasma was not platelet poor. APLA titers at the time of an acute thrombotic event may be decreased temporarily, thought to be due to consumption, but also may be transiently positive. Thus, the time of the acute thrombotic event is a suboptimal time for testing, and testing may better be delayed for a few weeks. Because APLA can be transient, guidelines suggest that repeatedly positive tests (at least 12 weeks apart) be documented, along with corresponding clinical phenomena, to confirm a diagnosis of APS.

A number of other APLA tests are not part of the revised Sapporo criteria, as their association with thrombosis or pregnancy loss has not been established, including immunoglobulin A (IgA) anticardiolipin and IgA anti- β_2 -glycoprotein I antibodies, antiphosphatidylserine antibodies, antiphosphatidylethanolamine antibodies, and antiphosphatidylinositol antibodies. There is presently no clear indication for testing for these additional APLAs in routine clinical practice. The different anticardiolipin and anti- β_2 -glycoprotein I antibody test kits available for clinical practice are not standardized. Also, lupus anticoagulant reporting is not standardized, and laboratory reports can be difficult to read and interpret. Thus, familiarity with the methods of a particular laboratory is especially desirable for APLA testing.

The INR determined from plasma occasionally is invalid in APS patients on VKAs because of a lupus anticoagulant effect on the prothrombin time. Furthermore, for patients with APLAs, INR determinations by point-of-care INR monitors are often inaccurate and significantly overestimate a patient's level of anticoagulation, thereby putting the patient at risk of recurrent thrombosis. Alternative tests, such as chromogenic factor X activity, can be used to measure the VKA effect when laboratory-based or point-of-care INR testing may be inaccurate. The target ranges for these tests depend on the reagents and instruments used for their determination, but an INR range of 2.0 to 3.0 typically corresponds to a chromogenic factor X activity of approximately 20% to 40%.

Risk for thrombosis

Positivity for all 3 APLA tests (ie, lupus anticoagulant, anticardiolipin, and anti- β_2 -glycoprotein I antibody tests; so-called triple positive) is associated with the highest risk for both venous and arterial thrombosis (and pregnancy loss). Patients with APS are thought to be at high risk of recurrent thrombosis, but the degree to which the recurrence risk is increased (compared to a similar patient who tests negative for APLA) is not well established. There is a 5% to 15% failure rate of warfarin therapy in preventing recurrent thrombosis in patients with APS.

Management

Because of the previously mentioned challenges related to laboratory APLA testing and interpretation, as well as the transient nature of antibodies in many patients, it is advisable to always question a diagnosis of APS until the previous laboratory test results have been reviewed and, if necessary, repeat testing has been performed. Because of the high rate of recurrent VTE, patients with APS with a history of unprovoked VTE should be maintained on anticoagulation indefinitely. Randomized trials have shown that a target INR range of 2.0 to 3.0 is equally effective in preventing recurrent thrombosis as a target range of 3.0 to 4.0. This probably holds true as long as the INR is reliable and indicates a patient's true level of anticoagulation. If the aPTT is prolonged at baseline due to a lupus anticoagulant, then anti-factor Xa levels need to be used to monitor heparin therapy. If the PT is prolonged at baseline, then the validity of the patient's INR should be checked once the patient is on VKA by comparing the INR to a chromogenic factor X assay. It then can be determined whether the INR is a reliable measure of that patient's anticoagulation and can be used for VKA monitoring. Also, if whole blood point-of-care (POC) INR testing is planned for a patient with APS, results of the POC instrument should be correlated with venipuncture plasma-based INR results tested in the clinical laboratory. As APLA titers can fluctuate over time, a recorrelation between the INR measured by POC and from a phlebotomy plasma sample should be performed every so often, such as every 4 to 6 months. It is not known, however, what the optimal frequency of such recorrelation is. Whether the DOACs are more, less, or equally effective compared to VKAs in patients with APS is currently being studied. It is likely that in patients with more common phenotypes of APS (ie, a single episode of VTE), a DOAC is effective, as such patients (albeit not identified as such) have also been included in the clinical trials assessing the efficacy and safety of these agents compared to LMWH/VKA.

It is not known whether patients with arterial thrombosis and APS are more effectively treated with antiplatelet or VKA anticoagulation therapy. Some evidence, from patients with APLA and noncardioembolic stroke, suggests that acetylsalicylic acid and VKA therapy may be equally effective. In the absence of prospective randomized trial data, this clinical question remains unanswered. Rituximab has been shown to decrease APLA titers in some patients, but whether lowering (or spontaneous disappearance) of APLA leads to a decreased thrombosis risk is not known. The management of pregnant women with APLA is discussed elsewhere in this self-assessment program.

Other thrombophilias

Lipoprotein(a)

Lipoprotein(a) [Lp(a)], which is involved in cholesterol metabolism, competes with plasminogen for binding to fibrin because of its structural similarity with plasminogen. This impairs plasminogen activation, plasmin generation, and fibrinolysis. Lp(a) also binds to macrophages and promotes foam-cell formation and the deposition of cholesterol in atherosclerotic plaques. Elevations in Lp(a) are associated with coronary heart disease and stroke in adults, as well as ischemic stroke in children. Individual studies in adults have not shown consistent association between elevated Lp(a) and the risk of either first or recurrent VTE.

Factor VIII elevation

General information

Elevated plasma levels of factor VIII are an independent and dose-dependent risk factor for VTE. Elevations in factor VIII have a familial-inherited component, but they do not follow a simple Mendelian inheritance pattern. Among first-degree relatives of patients with thrombosis and persistently elevated levels of FVIII, 40% had elevated levels of FVIII.

Prevalence

Elevated factor VIII levels have been defined operationally as values found in the top decile of a given population. Factor VIII is an acute-phase reactant, and baseline levels vary considerably. In the population-based Leiden thrombophilia study, 25% of patients with a first episode of VTE had elevations in factor VIII without elevations in C-reactive protein. Elevations in factor VIII are seen commonly in patients of African ancestry with VTE.

Laboratory aspects

Factor VIII clotting (functional) assays are available but have not been standardized to define the top decile of the local reference population.

Risk for thrombosis

Population-based, controlled studies have demonstrated that elevations in factor VIII >150% confer a 4.8-fold greater risk for first-episode VTE than if levels are <100%. In a large family study of first-degree relatives of patients with VTE or premature arterial disease and elevated levels of FVIII, the absolute annual incidence in the youngest age group with elevated levels of FVIII:C was 0.16% (CI 95%, 0.05% to 0.37%) and gradually increased to 0.99% (CI 95%, 0.40% to 2.04%) in those older than

60 years of age, although the odds ratios were not statistically significant.

Some studies have shown that elevated factor VIII levels are also a risk factor for recurrent VTE, but this has not been found uniformly.

Management

The role of elevated factor VIII levels in recurrent VTE is controversial. Since factor VIII is an acute phase reactant, its levels can vary substantially over time; furthermore, there is no consensus about the level of factor VIII activity at which a meaningful increase in recurrence risk would be seen. For patients with unprovoked VTE who are contemplating discontinuation of anticoagulant treatment, a FVIII activity determination may be reasonable if a very high value (>200% to 250%) would lead them to remain on treatment that they otherwise would have discontinued. However, as discussed in the “Duration of anticoagulation” section of this chapter, the most important clinical predictor of recurrence risk is the nature (provoked vs unprovoked) of the original thrombotic event.

Homocysteine and MTHFR

General information

Homocystinuria is a rare autosomal recessive defect in the homocysteine pathway, most commonly in the cystathione- β -synthase enzyme and is associated with markedly elevated homocysteine levels (>100 μ M/L). Based on newborn screening, the worldwide prevalence of cystathione- β -enzyme deficiency is reported at 1 in 344,000 live births. Affected individuals have a high rate of arterial and venous thrombotic events before the age of 30 years. A number of associated symptoms and signs occur, most commonly dislocation of the lens. On the other hand, mild to moderately elevated homocysteine levels are common and are referred to as hyperhomocysteinemia. Elevated levels may be due to deficiency of vitamin B₆, vitamin B₁₂, or folate; renal impairment; polymorphisms in the genes involved in the synthesis of the enzymes of the homocysteine metabolism; or unknown causes.

Modestly elevated levels of plasma homocysteine have been shown to be associated with an increased risk of venous and arterial thrombosis. However, a number of prospective, controlled studies have demonstrated that lowering a patient's homocysteine level does not decrease the risk of either first or recurrent thromboembolism (venous or arterial). The methylenetetrahydrofolate reductase (MTHFR) enzyme is a regulator of homocysteine metabolism. Polymorphisms in the *MTHFR* gene may lead to elevated plasma homocysteine levels, but do not necessarily do so.

Prevalence

A common *MTHFR* mutation is the C677T or “thermolabile” mutation, for which approximately 34% to 37% of US whites are heterozygous and 12% are homozygous. The A1298C polymorphism occurs in most ethnic groups and is present in the heterozygous state in 9% to 20% of the population. Elevated homocysteine levels may be seen in an individual with homozygous C677T mutation or double heterozygous C677T plus A1298C mutation but also may occur in the absence of these polymorphisms.

Risk for thrombosis

Meta-analyses show that the *MTHFR* polymorphisms in North America, where food is supplemented with folic acid, are not risk factors for venous and arterial thromboembolism or for pregnancy complications.

Management

Because the presence of *MTHFR* polymorphisms is not a thrombophilic state, there is no indication to test for these mutations. Because lowering of homocysteine levels has no demonstrated clinical benefit on thrombotic risk, there is no indication for treatment of elevated homocysteine levels with B vitamin or folic acid supplementation. Finally, because finding elevated homocysteine levels has no clinical consequences, there is no rationale to routinely measure homocysteine levels in thrombophilia evaluations. The exception may be in the younger individual (<30 years of age) with arterial thromboembolism or VTE in whom there is a suspicion for homocystinuria.

Others

Thrombosis may occur as a complication of systemic or local infection. Head and neck infections may trigger CSVT. Liver disease not only leads to a coagulopathy with bleeding diathesis due to decreased synthesis of procoagulant factors but also can lead to an increased risk for thrombosis because of decreased synthesis of anti-coagulants (eg, AT, protein C, and protein S) and fibrinolytic factors. In children, complex congenital heart disease is highly associated with both venous and arterial thrombotic events, either because of the disorders or the need for cardiac catheterizations, hospitalization, and major surgeries.

Thrombophilia: reasons to test or not test

Thrombophilia testing often is considered for patients who (i) experience unprovoked VTE at a young age (<50 years), (ii) experience unprovoked thrombosis at an

unusual site, (iii) have a history of VTE in 1 or more first-degree relatives, and (iv) remain uncertain about whether to continue anticoagulant therapy after estimating the risk of recurrence with other available information (sex, post-treatment D-dimer concentration, family history).

A variety of reasons for and against thrombophilia testing exist (Table 9-9). Importantly, negative thrombophilia testing does not necessarily correlate with a low risk of VTE recurrence. In asymptomatic relatives, the presence of inherited thrombophilia may alter decisions regarding contraceptive measures or postpartum prophylaxis in young women. An important requisite is that a test result indeed dichotomizes carriers and noncarriers in terms of their risk for a first episode of VTE. For women who wish to use oral contraceptives and who have a positive first-degree relative with VTE and a known thrombophilic defect, one can estimate the effect of avoidance of oral contraceptives on the number of prevented episodes of VTE by means of thrombophilia testing; or alternatively, by using a positive family history without thrombophilia testing. The results are listed in Table 9-6, in which the first column shows the observed incidence of VTE during 1 year of oral contraceptive use in carriers and noncarriers from thrombophilic families. From the risk difference between carriers and noncarriers (second column) the number of women that need to refrain from oral contraceptive use to prevent 1 episode of VTE can be calculated (third column). Table 9-6 clearly indicates that women with AT, protein C, or protein S deficiency

have a high absolute risk of VTE provoked by use of oral contraceptives. However, in these families, women *without* a deficiency also have a markedly increased risk of oral contraceptive-related VTE compared to pill users from the general population (0.7% vs 0.04% per year of use), reflecting a selection of families with a strong thrombotic tendency in which yet-unknown thrombophilias have cosegregated. Thus, although selective avoidance of oral contraceptive use prevents VTE episodes in deficient women, for women from these families a negative thrombophilia test may lead to false reassurance.

In the 2012 ACCP guidelines, the absence or presence of thrombophilia did not influence recommendations on duration of anticoagulant therapy in patients with VTE because thrombophilias as a group were assessed to be not strong or consistent enough risk factors to meaningfully predict recurrence of VTE. The United Kingdom-based National Institute for Clinical Excellence (NICE) guidelines recommend that hereditary thrombophilia testing be considered “in patients who have had unprovoked DVT or PE and who have a first-degree relative who has had DVT or PE if it is planned to stop anticoagulation treatment.” The same guideline suggests that APLA testing be done only “in patients who have had unprovoked DVT or PE if it is planned to stop anticoagulation treatment.” ASH is developing guidelines on the topic of thrombophilia testing. Should the decision to test for thrombophilia be made, Table 9-10 provides guidance toward the specific tests in adults and children.

Table 9-9 Reasons for and against thrombophilia testing

Reasons for testing
Patient with thrombosis
Influence on duration of anticoagulation therapy
Possible explanation (for patient and physician) why thrombosis occurred
Reasons against testing
Lack of therapeutic consequences even if test positive/abnormal
Suboptimal performance of tests (false-positive and false-negative results) or misinterpretation of tests
Poor medical advice based on test results
Anxiety, if test is positive
False sense of security that thrombosis risk is low, if test result normal/negative
Cost of testing
Lack of impact for asymptomatic first-degree relatives (possible exception is women contemplating estrogen use or pregnancy)
Impact on ability to obtain life or health insurance

Inherited and acquired thrombophilia in neonates and children

The inherited thrombophilias are by definition present throughout childhood, yet for the most part remain asymptomatic. However, the diagnosis of heterozygous deficiencies of the plasma inhibitors (AT, protein C, and protein S) is difficult due to the reduced levels of these proteins attributable to developmental hemostasis. Comparison of results to age-appropriate reference ranges is critical. In contrast, while rare, the presentation of homozygous deficiency of protein C or S as neonatal purpura fulminans is one of the most dramatic hematological presentations in childhood.

Homozygous or double-heterozygous protein C deficiency is associated with catastrophic thrombotic complications at birth, manifested by neonatal purpura fulminans (extensive microvascular thrombosis of the skin) and, less commonly, massive DVT. Approximately 70% of affected infants have CNS or retinal infarction prior to birth. For confirmation of homozygous protein C deficiency in a neonate with purpura fulminans or massive venous

thrombosis, the infant should have undetectable protein C activity (<5 IU/dL) and both parents should be heterozygous for protein C deficiency. Treatment options include high intensity anticoagulation (usually warfarin), or replacement with plasma-derived protein C concentrates or a combination of both. Central vascular access should be avoided due to the high rates of thrombotic complications. Protein C infusions can be given subcutaneously. Neonates and children with severe inherited protein C deficiency have an ongoing risk of purpura fulminans, and therefore require long-term therapy. Liver transplantation may be a cost-effective option. Purpura fulminans can occur in the rare newborn with severe protein S deficiency because of homozygous or double heterozygous mutations. The management principles of purpura fulminans are similar to homozygous protein C deficiency, except that no protein S concentrate exists. Therefore, fresh frozen plasma (FFP) is the treatment of choice.

When thinking about thrombophilia in children, one must remember that the single most important risk factor for VTE in neonates and children is the presence of a CVAD, highlighting the roles of blood flow and endothelial damage in VTE pathogenesis in children. Of note, large kindred studies that traced the natural history of inherited thrombophilias reported that the age of first thrombosis is usually in the third or fourth decade of life, and thrombosis during childhood is rare. The results of single studies on the risk of VTE onset and recurrence associated with inherited thrombophilia in children are contradictory or inconclusive, mainly due to lack of statistical power, and often the lack of rigorous prospective cohort study designs. Studies of natural anticoagulants are also hampered by developmental hemostasis, with the normal plasma concentrations of these proteins changing with age, often quite markedly during childhood; and these physiological changes are often not considered adequately in published papers in the classification of children as deficient.

In 3 systematic reviews and meta-analyses, including observational studies in pediatric patients with VTE and cerebrovascular occlusion (cerebral venous thrombosis and stroke) more than 70% of patients had at least 1 clinical risk factor. The pooled odds ratios showed statistically significant associations between FVL, prothrombin 20210 mutation, protein C, protein S, or AT deficiency, elevated Lp(a), combined thrombophilia and the presence of acquired lupus anticoagulant/APLAs and VTE onset. The pooled odds ratios for VTE onset ranged from 2.4 for the prothrombin mutation to 9.4 in children with AT deficiency. In addition, the pooled odds ratio with respect to persistent APLAs/lupus anticoagulant was 6.6 for cere-

brovascular venous occlusion and 4.9 for VTE. However, these systematic reviews were based on observational studies that mostly had poor design. There was no uniformity of thrombophilia testing or definition, prospective follow-up, or standardization of outcome ascertainment. Patient subgroups like provoked or unprovoked VTE, neonatal VTE, central-line related VTE, and malignancy-related VTE could not be analyzed separately since they (i) were too small, (ii) were not clearly defined or fully separated in the original studies, and (iii) could not be clarified retrospectively by the author groups contacted at the time of performing the meta-analyses.

Interestingly, in the highest quality study to date, which was subsequent to the aforementioned meta-analysis, Curtis et al performed a prospective, population-based, controlled, disease-specific study that suggests minimal association between perinatal stroke and thrombophilia (specifically a broad range of thrombophilia markers). The authors make the relevant point that this does not exclude a role for disordered coagulation in the etiology of the event, but that such a role is unlikely to be found by testing standard thrombophilia assays.

The rationale for thrombophilia testing in children in terms of outcomes or alterations to duration or intensity of treatment remains dubious. Thrombophilia testing is frequently performed because clinicians, in an attempt to provide some answers for desperate parents, embark on testing knowing that the interpretation of any positive results is fraught with uncertainty. Alternatively, testing is often driven by parents who have been scouring the internet for answers and come asking about thrombophilia. At present, consensus recommendations suggest that thrombophilia testing (AT, protein C, protein S, factor V Leiden, prothrombin gene mutation, and lupus anticoagulant/APLAs) may be appropriate in children with unprovoked and recurrent VTE. Some reports suggest that anatomical abnormalities (eg, absent IVC, thoracic outlet syndrome) are more likely to be the cause of spontaneous VTE in children and adolescents than a plasma-derived thrombophilia, and that careful imaging is required. There seems to be no role for thrombophilia testing in neonates, infants, and children with provoked VTE, especially asymptomatic or symptomatic central line-related VTE. The role of testing nonsymptomatic siblings and further first-degree family members in high-risk families with known AT-, protein C-, or protein S-deficiency carriers, and in individuals with a first-degree family history of unprovoked young-onset VTE is uncertain, but it would seem that adolescents, especially females, have the most to gain from such testing.

APLA can be found in a high percentage of children without any underlying disorder, with an estimated frequency that ranges from 3% to 28% for anticardiolipin antibodies and from 3% to 7% for anti- β_2 -GPI antibodies. The reason for such frequent occurrence in comparison with adults has been related to the frequent exposure of children to infectious processes. The majority of these antibodies are transient and disappear within a few weeks to few months (~3 to 6 months). Studies of healthy children who present for surgery, especially tonsillectomy, show a 2% prevalence of transient lupus anticoagulant with no apparent pathologic consequence due to the fact that these postinfectious APLAs more commonly bind cardiolipin in a non- β_2 -glycoprotein-I-dependent manner. The prognostic significance of the transient lupus anticoagulant in children who present with thrombosis in the setting of concurrent infection is probably similar to that of children who have an asymptomatic lupus anticoagulant. It is difficult to estimate the prevalence of APS in the pediatric population because there are no validated criteria, and the diagnosis rests on extension of adult guidelines and clinical judgment. Transplacental transmission of maternal APLA has been reported in the newborn period. Registry data suggest that these antibodies are not associated with thromboembolic events.

There are a number of other acquired thrombophilic states in children. Acquired AT deficiency secondary to asparaginase chemotherapy or nephrotic syndrome has been implicated in the pathogenesis of increased thrombosis in these patients; however, AT supplements have not been shown to be beneficial. In recent years, administration of AT has become popular in children requiring UFH therapy, especially those on extracorporeal membrane oxygenation (ECMO). This is predicated on the untested theory that because many sick children have acquired AT deficiency (and young infants have naturally lower AT levels), providing more substrate for heparin to bind facilitates a reduction in heparin requirements and more effective anticoagulation. In most cases, this therapy involves increasing AT to significant supraphysiological levels for the individual child. Data supporting this practice are sparse. The reduction in heparin requirements following AT administration is highly variable and studies of children on ECMO have thus far either not examined or not shown any beneficial effect of AT on clinical outcomes—including bleeding, blood product administration, ECMO circuit changes, length of stay, or mortality. Further, AT is used to assist achievement of therapeutic target ranges for UFH (whether using activated clotting time, aPTT or anti-Xa factor as the monitoring test) that in fact have never been

proven to be optimal in any comparative trial. In other groups of hospitalized children, such as those in the neonatal ICU or the general ward, AT administration may be either ineffective or harmful.

A large analysis of AT administration in children on ECMO reported 8,972 children who received ECMO in 43 hospitals across the United States over a decade; 1,931 (21.5%) of whom had received at least 1 dose of AT during their ECMO run (predominantly early in the ECMO course). AT use varied between hospitals from 0% to 80% but increased over the course of the study, from approximately 2% in 2005 to 50% by 2012. The children who received AT were more likely to be younger, smaller, and have chronic conditions. AT administration was associated with a higher incidence of thrombosis (OR 1.55; 95% CI, 1.36 to 1.77), including pulmonary embolus and ischemic stroke, and a higher incidence of hemorrhage (OR 1.27; 95% CI, 1.14 to 1.42), including central nervous system hemorrhage. There was no difference in mortality. Routine use of AT supplementation in children requiring UFH for ECMO or any other reason is difficult to justify.

In general, the rates of thrombosis in children with cancer are much lower than those seen in adults, and vary according to cancer type. In the absence of CVADs or direct venous compression, thrombosis in children with cancer is less common. Myeloproliferative diseases and PNH are rare in children, but if they do occur, can be associated with thrombosis. “Particularly, children with acute lymphoblastic leukemia who are treated with asparaginase therapy have a risk of venous thromboembolism of approximately 10.”

Table 9-10 Thrombophilia tests to consider if decision to test for thrombophilia is made

Venous thromboembolism
Factor V Leiden mutation
Prothrombin 20210 mutation
Protein C activity
Protein S activity, free protein S antigen
Antithrombin activity
Anticardiolipin IgG and IgM antibodies
Anti- β_2 -glycoprotein I IgG and IgM antibodies
Lupus anticoagulant
Hemoglobin, platelet count, JAK2V617F and PNH (in splanchnic vein thrombosis)
Lipoprotein(a) (in pediatrics)
Arterial thromboembolism, unexplained
See Table 9-2
PNH, paroxysmal nocturnal hemoglobinuria.

Interpreting test results and educating patients

When interpreting thrombophilia laboratory test results, it is important to be aware of the circumstances that lead to abnormal test results without a true thrombophilia being present. Several results are temporarily abnormal in the patient with acute thrombosis and therapy with heparin and VKAs (Tables 9–7 and 9–8). As a general principle, counseling should come before the decision for laboratory testing is made and tests performed. In our experience, inappropriate testing in adult practice, at least in the United States, is common. When a thrombophilia is identified, educating the patient and the patient's family members is important. Online education and support resources on a variety of thrombophilias and the genetic aspects of family testing exist (eg, see <http://www.clotconnect.org> or <http://www.stoptheclot.org>).

Antithrombotic drugs

Anticoagulants

Heparins

Mechanism of action

Heparins are extracted from porcine intestine or bovine lung and consist of glycosaminoglycans of different lengths. UFHs have a mean length of 40 monosaccharide units. LMWHs are made from UFH through chemical and physical processes and have a mean length of 15 monosaccharide units. A pentasaccharide structure within these polysaccharide molecules binds to and enhances the action of AT, which inactivates thrombin and factor Xa. Molecules of 18 monosaccharide units or more are required to bind thrombin and AT simultaneously (ie, to enhance heparin's AT effect on thrombin). The 5 sugars of the pentasaccharide structure, however, are sufficient to lead to a conformational change of AT that can then inactivate factor Xa. Therefore, LMWHs inactivate mostly factor Xa, whereas UFH acts against thrombin and factor Xa. Fondaparinux is a synthetic pentasaccharide that binds to AT, leading to specific inactivation of factor Xa.

Unfractionated heparin

UFH at therapeutic doses is given through continuous intravenous infusion and is typically monitored using aPTT. The therapeutic aPTT range depends on the heparin sensitivity of the aPTT reagent and the instrument used by a laboratory. A therapeutic aPTT is considered that which corresponds to a plasma anti-Xa heparin level of 0.3 to 0.7 U/mL. Optimally, a coagulation laboratory should provide clinicians with the therapeutic aPTT range for the reagent-instrument combination used in that labo-

ratory. If a laboratory has not provided a therapeutic aPTT range for aPTT determinations, then an aPTT ratio of 1.5 to 2.5 times the midpoint of the normal range is often considered to be therapeutic. With some aPTT reagents, however, this range is subtherapeutic and underdosing of a patient may occur.

UFH therapy also can be monitored with anti-Xa levels, and a number of laboratories have switched to routinely using this method for UFH monitoring. Although this is an acceptable alternative, it is not known which method leads to superior safety or efficacy of heparin therapy. UFH is mostly cleared by the reticuloendothelial system and to a smaller degree by the kidney. The half-life of heparin in plasma depends on the dose given. It is 60 minutes with a 100 U/kg bolus. A patient on continuous infusion intravenous UFH at therapeutic doses likely will have a return to the baseline aPTT within 3 to 4 hours after discontinuation of heparin.

Weight-based heparin-dosing nomograms achieve therapeutic aPTTs faster than other approaches to selecting a UFH dose. In many patients at average risk for bleeding, a loading dose of 80 U/kg of intravenous heparin, followed by a continuous infusion of 18 U/kg/h is appropriate for full anticoagulation. This dosing, however, may have to be modified in the patient at higher risk for bleeding. The aPTT or anti-Xa level should be determined 6 hours after initiation of heparin and each dose change, and once every 24 hours once the aPTT or anti-Xa level is in the therapeutic range. In the occasional patient in whom the aPTT is invalid, such as a patient with a lupus anticoagulant, anti-Xa levels need to be used for heparin monitoring. Long-term use of UFH leads to an increased risk of osteoporosis and carries a risk for heparin-associated thrombocytopenia.

UFH remains a commonly used anticoagulant in pediatric patients. In tertiary pediatric hospitals, approximately 15% of inpatients are exposed to UFH each day. There are a number of specific factors that may alter the effect of UFH in children (Table 9–11). The clinical implications of these changes on dosing, monitoring, and the effectiveness/safety profile of UFH in children remains uncertain.

There have been no reported clinical outcome studies to determine the therapeutic range for UFH in neonates or children, so the therapeutic range for all indications is extrapolated from those used in VTE therapy in adults. This equates to an aPTT that reflects a heparin level by protamine titration of 0.2 to 0.4 U/ml or an anti-factor Xa level of 0.3 to 0.7 U/ml. There are multiple reasons why this extrapolation might be invalid; however, the safety and efficacy of this approach, in experienced hands, seems reasonable.

Table 9-11 Factors in children which affect the action of UFH

UFH factor	Age-related difference
UFH acts via AT-mediated catabolism of thrombin and factor Xa	Reduced levels of AT and prothrombin Reduced capacity to generate thrombin Age-related difference in anti-Xa: anti IIa activity of UFH
UFH is bound to plasma proteins, which limits free active UFH	Alterations in plasma binding
Endothelial release of TFPI	Age-related differences in amount of TFPI release for same amount of UFH

AT, antithrombin; TFPI, tissue factor pathway inhibitor; UFH, unfractionated heparin.

Bolus doses of 75 to 100 U/kg result in therapeutic aPTT values in 90% of children at 4 to 6 hours postbolus. Maintenance UFH doses are age dependent, with infants (up to 2 months) having the highest requirements (average 28 U/kg/hr) and children over 1 year having lower requirements (average 20 U/kg/hr). The doses of UFH required for older children are similar to the weight-adjusted requirements in adults (18 U/kg/hr). However, boluses of 75 to 100 U/kg in children have been shown to result in excessive prolongation of aPTT for over 100 minutes, implying that the recommendations may need to be re-examined. In many cases, especially where bleeding risk is higher, therapy should be commenced with an infusion only, and no boluses. Reduced doses are usually required in renal insufficiency.

Monitoring of UFH therapy is current standard practice, but there are difficulties with interpreting the monitoring assays, related to a lack of correlation between anti-Xa, aPTT, and thrombin clotting time, as well as in making dosage adjustments. The ratio of anti-Xa to IIa effect changes with age and dose, and the half-life of UFH also varies with age. There are no published studies in children that establish the ideal frequency of UFH monitoring, and vascular access is a frequent limiting factor. Contamination of results when blood is taken from the same limb into which the infusion is being given is often a major issue. Many experienced clinicians use small incremental changes and no boluses to feel comfortable about monitoring on a once-daily basis, which is often more practical. Given that there are no data to support the absolute advantage of a defined therapeutic range, and if one takes into account the rationale for treatment and the clinical progress of the patient in decision making, then this seems a reasonable approach.

Further studies are required to accurately determine the frequency of UFH-induced bleeding in optimally treated children, which is probably below 1%, depending on patient selection and experience of the managing team. Probably the most common cause of fatal bleeding secondary to UFH relates to accidental overdose, especially in neonates. While rarely reported in the medical literature, the number of deaths reported in the popular press appears to be increasing. This often occurs in children who are receiving low-dose UFH flushing of vascular access devices, intended for example to be 50 U/5 ml UFH. Errors in vial selection and failure of bedside checking procedures result in 5000 U/5 ml UFH being injected, and in small infants this results in a massive and unexpected overdose of UFH. Units should actively manage the choices of UFH preparations available to their staff to minimize the risk of confusion. Staff should be educated in the dangers of UFH and encouraged to be vigilant at all times when administering a drug that consistently ranks in hospital lists of the drugs most commonly involved in medication errors. Rapid reversal of UFH can be achieved with protamine titration, although in many instances, simple cessation of UFH infusion is adequate.

Low-molecular-weight heparin

The various LMWH drugs differ in their composition, and thus in their degree of inhibition of thrombin and factor Xa. Therefore, dose recommendations for VTE prophylaxis and for full-dose treatment vary for the various LMWHs. The lack of significant binding of LMWHs to plasma proteins gives them a more predictable anticoagulant effect than UFH, so that fixed or weight-adjusted dosing is possible without the need for routine anticoagulant laboratory monitoring. The peak plasma effect is reached 3 to 4 hours after injection. The half-lives of the various agents differ, ranging between 3 and 7 hours. Once- or twice-daily dosing regimens are available for the different drugs. Since the LMWHs are—to varying degrees—renally cleared, anti-Xa activity measurement at steady state is suggested in patients with renal impairment. Because the pharmacokinetic effect of impaired renal function differs among LMWHs, however, there is not a single creatinine clearance cutoff value below which dose reduction or assessment of anticoagulant effect is needed. Below a glomerular filtration rate of 30 mL/min, caution with LMWH dosing is appropriate and reference to the package insert for the individual LMWH being used appears advisable to determine FDA recommendations on dose. In severe renal impairment (creatinine clearance <15 mL/min) and dialysis dependence, UFH should be chosen over LMWH. It may be appropriate to increase the prophylactic dose of LMWH for patients with morbid obesity (body mass

index of $>35 \text{ kg/m}^2$). For full-dose LMWH use, dosing should be based on actual body weight, and anti-Xa measurement generally is not necessary for patients weighing up to 150 kg. Anti-Xa activity measurement and twice-(rather than once-) daily dosing should be considered in patients with morbid obesity.

An expected anti-Xa level (obtained 3 to 4 hours after subcutaneous injection) is in the order of 1.0 to 2.0 U/mL for once-daily dosing; for twice-daily dosing, it is 0.6 to 1.2 U/mL. Anti-Xa levels might be useful if a patient on LMWH has a recurrent thrombosis or a significant bleed to document whether the patient had sub- or supratherapeutic anti-Xa levels, which could explain the clotting or bleeding event. Anti-Xa activity might also be advisable in patients using LMWH in the setting of severe renal impairment. That being said, neither “high” nor “low” levels of anti-Xa activity have been well correlated with the risk of adverse clinical outcomes.

LMWH has become the anticoagulant of choice in many pediatric patients for a variety of reasons. However, the predictability of the anticoagulant effect with weight-adjusted doses is lower than in adults, presumably due to differences in binding to plasma proteins. Table 9-12 provides guidance for dosing in children according to age. Most clinical data for LMWH in pediatric patients utilized enoxaparin.

Therapeutic ranges for LMWH are extrapolated from results in adults and based on anti-Xa levels; the guideline for subcutaneous administration twice daily being 0.50 to 1.0 anti-Xa U/mL at 2 to 6 hours following injection. Most studies in children have used this therapeutic range, although 1 study used a lower maximal level (0.8 U/mL)

Table 9-12 Therapeutic and prophylactic dosing of enoxaparin, tinzaparin, and dalteparin in children according to age

	Therapeutic dose	Prophylactic dose
Enoxaparin		
≤2 months of age	1.5 mg/kg SC b.d.	1.5 mg/kg SC o.d.
Tinzaparin	1 mg/kg SC b.d.	1 mg/kg SC o.d.
≤2 months of age	275 U/kg SC o.d.	75 U/kg SC o.d.
2–12 months of age	250 U/kg SC o.d.	75 U/kg SC o.d.
1–5 years	240 U/kg SC o.d.	75 U/kg SC o.d.
5–10 years	200 U/kg SC o.d.	75 U/kg SC o.d.
Dalteparin	175 U/kg SC o.d.	50 U/kg SC o.d.
>2 months of age	150 U/kg SC b.d.	150 U/kg SC o.d.
>2 months of age	100 U/kg SC b.d.	100 U/kg SC o.d.

SC, subcutaneously; b.d., twice daily; o.d., once daily.

with good efficacy and safety outcomes. Once-daily regimens are described much less commonly, and intravenous use has also been reported, but rarely. Reduced doses are required in renal insufficiency.

While initial doses most likely to attain the therapeutic range have been described, considerable interpatient dose differences exist, suggesting that routine monitoring of anti-Xa levels in children and neonates remains necessary. Monitoring protocols have been suggested. Whether clinical effectiveness is altered by having multiple age-related initial and maintenance dose recommendations is unclear. Recent studies have suggested even higher initial doses may be required for neonates to achieve therapeutic range, but given the absence of evidence that the therapeutic range extrapolated from adults is required in neonates, clinical outcome data would be more useful in driving changes to current therapy.

Major bleeding rates with LMWH in children appear to be low in stable patients, and although reports vary from 0% to 19%, patient selection is critical; and in many cases of bleeding, titratable and more readily reversible UFH would have been a better therapeutic option (eg, immediate postoperative patients). LMWH is only partially reversed by protamine. There are no data on the frequency of osteoporosis (although case reports exist in extended use of LMWH, especially in premature infants), heparin-induced thrombocytopenia (HIT), or other hypersensitivity reactions in children exposed to LMWH. Temporary hair loss is reported.

Fondaparinux

Fondaparinux is a synthetic pentasaccharide, is AT dependent, and consists of the 5 key monosaccharides of heparin that bind to AT and magnify AT-mediated inhibition of factor Xa. It is specific against factor Xa and does not inhibit thrombin. It is given subcutaneously, reaches its peak plasma level in 2 hours, and due to a half-life of approximately 17 hours, it is dosed once daily. Because it does not bind significantly to plasma proteins, it can be given without laboratory monitoring as a fixed dose for prophylaxis of VTE or in body weight-adjusted fashion for therapy of VTE. It is cleared by the kidney, and thus should not be used in patients with creatinine clearance $<30 \text{ mL/min}$. Fondaparinux does not cause (and is sometimes used to treat) HIT.

There are few data regarding fondaparinux in children. A single-arm, open-label, dose-finding, pharmacodynamic and safety study enrolled 24 patients aged 1 to 18 years and showed a dose of 0.1 mg/kg/d, mirroring the pharmacodynamic profile found in adults. It is recommended that children have therapeutic drug monitoring using a

fondaparinux-based anti-Xa assay. Peak levels should be measured at 3 hours after infusion, targeting a level of 0.5 to 1 mg/L (units are expressed as a concentration, but this is a unit conversion from the anti-Xa assay). In addition, for patients requiring procedures that are receiving fondaparinux, procedures should be performed at least 24 hours after the last dose. A multidose vial is not available, such that providing doses that are not available in prefilled syringes (2.5, 5, 7.5, and 10 mg) can be problematic.

Management of bleeding

If bleeding occurs in a patient on UFH, intravenous protamine can be given, which binds to and neutralizes heparin. Protamine can impair platelet function and interact with coagulation factors, causing an anticoagulant effect of its own. Therefore, the minimal amount of protamine to neutralize heparin should be given. LMWH is only partially reversed by protamine. In case of significant bleeding on LMWH, however, protamine should be considered. FFP likely has little, if any, effect on bleeding associated with heparin, LMWH, and fondaparinux and is not indicated unless there is also evidence of a coagulopathy resulting in factor depletion.

Heparin-induced thrombocytopenia

Heparin-induced thrombocytopenia is a rare but important complication that can occur with both UFH as well as LMWH administration. It is discussed in detail in Chapter 11. The true rate of HIT in children appears greatly reduced compared to that in adults.

Heparin resistance

Heparin resistance is a term used when patients require unusually high doses of UFH to prolong the aPTT into the therapeutic range or to prolong the activated clotting time above the value (typically >400 to 450 seconds) at which extracorporeal circulation on heparin is thought to be safe from an anticoagulant point of view. Causes include AT deficiency, increased heparin clearance, significantly low baseline aPTT (eg, due to elevations of factor VIII and fibrinogen), or increased nonspecific heparin-binding proteins.

Thrombin inhibitors

This section discusses only *parenteral* thrombin inhibitors; dabigatran, an oral thrombin inhibitor, is discussed in the section “Direct oral anticoagulants” in this chapter.

Hirudins

Natural hirudin is a 65-amino-acid direct thrombin inhibitor derived from the saliva of the leech *Hirudo medicinalis*. It does not require the presence of AT to exert its anticoag-

ulant effect. Several derivatives and recombinant products have been developed. Desirudin is also a 65-amino-acid recombinant hirudin, administered subcutaneously. Peak plasma levels are reached 1 to 3 hours after injection. It is metabolized primarily by the kidney, and dose reductions are needed in patients with moderate and severe renal impairment. It is FDA-approved for postsurgical VTE prophylaxis. Bivalirudin is a synthetic, 20-amino-acid polypeptide that directly binds to and inhibits thrombin. It is given intravenously and has a half-life of 25 minutes. Dose adjustment for severe renal impairment is necessary. It is FDA-approved for use during percutaneous transluminal coronary angioplasty, including patients undergoing it who have HIT.

Argatroban

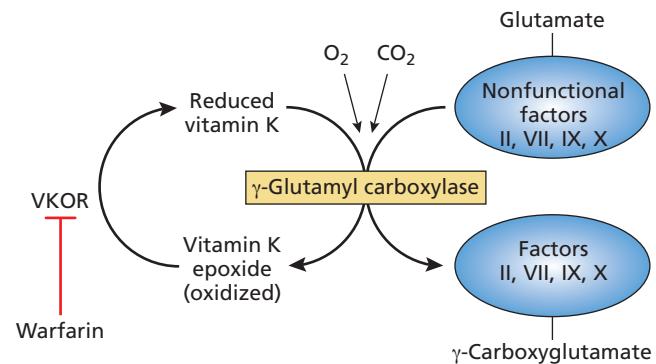
Argatroban is a small synthetic molecule that binds to and inhibits thrombin at its catalytic site. It is given intravenously. Since it is metabolized in the liver, dose reductions in patients with impaired liver function are necessary. Serum tests for liver function always should be obtained before its use. Its half-life is 40 to 50 minutes. The drug can be started without the need for an initial bolus. The dosing is adjusted to an aPTT of 1.5 to 3 times the initial baseline value (not to exceed 100 seconds). It is FDA-approved for the treatment of HIT.

Vitamin K antagonists

Mechanism of action

All coagulation factors are synthesized in the liver, although von Willebrand factor and factor VIII also are produced in extrahepatic sites. Factors II, VII, IX, X, protein C, and protein S need to be carboxylated in a final synthetic reaction to become biologically active. This step requires the presence of vitamin K (Figure 9-6). The half-lives of the vitamin K-dependent coagulation factors are 4 to

Figure 9-6 Role of vitamin K, point of activation of warfarin, and enzymes involved in vitamin K and warfarin metabolism.



6 hours for factor VII, 24 hours for factor IX, 36 hours for factor X, 50 hours for factor II, 8 hours for protein C, and 30 hours for protein S. Because of the long half-lives of some of these factors, particularly factor II, the full antithrombotic effect of VKAs is not reached until several days after having started these drugs. Because protein C has a relatively short half-life and decreases early, its lowering renders the patient hypercoagulable during the first few treatment days, before factor II, with its longer half-life, decreases and protects the patient from thrombosis. Thus, VKAs may create a paradoxical prothrombotic state in the first 5 days, putting the patient at risk for coumarin-induced skin necrosis and progression of thrombosis, unless a parenteral anticoagulant is given overlapping with the VKA in these first few days. The parenteral anticoagulant should be given for at least 5 days; thereafter it can be stopped when the INR is >2.0.

Monitoring and dose requirement

VKAs are monitored by prothrombin time, which is standardized between laboratories as an INR. Coumarin VKAs are metabolized by the cytochrome P450 enzyme complex, mostly the enzymes CYP2C9 and CYP1A2 (Figure 9-6). Because of a high degree of interindividual variability in the activity of these enzymes, there is a high degree of variability in the daily drug dose that patients need to maintain their INR in the narrow therapeutic range. Polymorphisms in the genes transcribing enzymes involved in the metabolism of VKAs, such as *CYP2C9* (cytochrome P2C9 enzyme) and *VKORC1* (vitamin K epoxide reductase complex-1) contribute to the interindividual variability in dose requirements. Finger stick (point-of-care) whole blood INR monitors are available and, up to an INR of 4.0, yield results comparable to plasma-based measurements performed on a laboratory-based instrument. INR home monitoring by appropriately selected patients is safe and effective and a good treatment option. In some patients with fluctuating INRs, daily supplementation with microdose oral vitamin K, such as 100 to 300 mg/d, has been shown to decrease INR fluctuations.

Available VKAs

Two classes of VKAs exist: coumarin derivates (warfarin, phenprocoumon, acenocoumarol, and tioclofarin), which are the most widely used VKAs; and the indandione derivatives (fluindione, anisindione, and phenindione), which are used in some countries outside the United States. The only FDA-approved VKAs are warfarin (approved in 1954) and anisindione (approved in 1957). Warfarin has a pharmacodynamic half-life of 1 to 2.5 days, with a mean of approximately 40 hours.

The typical loading dose of warfarin in the hospitalized patient is 5 mg daily on days 1 and 2, with subsequent dosing based on the INR measurement after the first 2 doses. In children, this equates to initial doses of 0.1 to 0.2 mg/kg. A frail or elderly patient, one who has been treated with prolonged antibiotics, has liver disease, or has undergone intestinal resection, needs a lower dose in the first few days. Women generally need lower doses. Some clinicians prefer using higher loading doses of 7.5 to 10 mg, particularly in a young, nutritionally replete outpatient. For maintenance dosing, the highest dose requirements for keeping a patient in the therapeutic range are in men <50 years old (median dose, 6.4 mg/d) and the lowest requirements are in women >70 years of age (median dose, 3.1 mg/d). Occasionally, patients need doses as high as 20 to 30 mg per day. Genetic testing for polymorphisms of the *CYP2C9* and *VKORC1* enzyme genes is available and helps predict, to some degree, warfarin doses needed to reach therapeutic INR ranges; but despite extensive clinical trial testing, pharmacogenetic testing has not been shown to reduce the risk of thrombosis or bleeding.

Management of elevated INRs and bleeding

Several options exist to manage elevated INRs and bleeding that occur on VKAs, depending on the degree of INR elevation and the presence or absence of risk factors for bleeding and of active bleeding itself. A general management strategy is presented in Table 9-13 and encompasses holding the next anticoagulant dose(s) and giving vitamin K. Giving too high a dose of vitamin K should be avoided if there is no major bleeding, because it reverses the INR completely and may make re-anticoagulation more difficult. FFP can lower the INR to an extent, but not completely or markedly because the amount of any particular clotting factor in a unit of plasma is small. If complete or immediate INR reversal is needed, such as when treating a major bleeding episode, a prothrombin complex concentrate (PCC) is preferred over FFP, if available. PCCs are plasma-derived products from human donors that contain high concentrations of the vitamin K-dependent factors (ie, II, VII, IX, and X). They exist as so-called 4-factor PCCs containing all vitamin K-dependent coagulation factors, and as 3-factor PCCs, which contain relatively low concentrations of factor VII. The 4-factor products are capable of restoring individual clotting factor activity to nearly 100% within minutes of administration of a low-volume intravenous infusion. KCenta is the only 4-factor PCC available in the United States as of February 2015. Recombinant factor VIIa is not recommended in the management of VKA-associated hemorrhage.

Table 9-13 Management strategy for elevated INRs in patients on VKAs

INR	Bleeding?	Risk factors for bleeding?	Intervention
Supratherapeutic, but <5.0	No	No/yes	Lower or omit next VKA dose(s); reduce subsequent dose(s)
5.0–9.0	No	No	Omit next VKA doses; reduce subsequent dose; low-dose oral vitamin K accelerates INR drop but is not likely to improve clinical outcome
5.0–9.0	No	Yes	Vitamin K 1–2.5 mg orally
>9.0	No	No/yes	Vitamin K 2.5–5 mg orally
Serious bleed at any INR	Yes		Vitamin K 10 mg iv + FFP or PCCs

FFP, fresh frozen plasma; INR, international normalized ratio; iv, intravenously; PCCs, prothrombin complex concentrates; VKA, vitamin K antagonist.

Table 9-14 Recommendations when interrupting warfarin therapy for invasive procedures*

Risk of thrombosis	Before surgery	After surgery
Low	d/c warfarin 5 d preop No LMWH or low-dose LMWH	Restart warfarin 12–24 h after surgery No LMWH or low-dose LMWH
Intermediate	d/c warfarin 5 d preop No LMWH or low-dose LMWH	Restart warfarin 12–24 h after surgery No LMWH or low-dose LMWH
High	d/c warfarin 5 d preop Full-dose LMWH or iv UFH	Restart warfarin 12–24 h after surgery Full-dose LMWH or iv UFH

*These recommendations are “grade C” recommendations (ie, very weak recommendations based on little or no high-quality evidence).

Other alternatives may be equally reasonable.

d/c, discontinue; iv, intravenous; preop, preoperatively; LMWH, low-molecular-weight heparin; UFH, unfractionated heparin.

Periprocedural interruption of VKA therapy

Whether there is a need to stop oral anticoagulant therapy before a surgical or radiological procedure depends on the bleeding risk associated with the procedure. How far in advance of the procedure to stop VKAs depends on the INR, the age of the patient, and the half-life of the VKA. Bridging therapy with a subcutaneous or intravenous anti-coagulant is typically unnecessary but may be beneficial in patients whose thrombosis risk is very high (Table 9-14).

Pediatric considerations

Warfarin is the most commonly used and studied VKA in children worldwide. Acenocoumarol is administered with high frequency in some European and South Ameri-

can countries, and phenprocoumon is the preferred VKA in some parts of Europe. The current therapeutic INR ranges for children are extrapolated from recommendations for adult patients, because no clinical trials have assessed the optimal INR range for children. For most indications, the therapeutic target INR is 2.5 (range 2.0 to 3.0), although the therapeutic ranges for prosthetic valves varies according to type and position.

Warfarin is usually commenced at 0.1 to 0.2 mg/kg, capped at 5 mg maximal starting dose. Patients with liver impairment, or post-Fontan surgery, require lower doses.

Monitoring oral anticoagulant therapy in children is difficult and requires close supervision with frequent dose adjustments. Only 10% to 20% of children are safely monitored monthly. Studies in children comparing POC monitors to venipuncture INR confirm their accuracy and reliability. The major advantages of POC devices include reduced trauma of venipunctures, minimal interruption of school and work, ease of operation, and portability. However, all POC devices are operator dependent and considerable family education is required to ensure accurate use, and an ongoing quality assurance program is recommended.

VKAs are often avoided in infants for several reasons:

- The plasma levels of the vitamin K-dependent coagulation factors are physiologically decreased in comparison with adult levels.
- Infant formula is supplemented with vitamin K to prevent hemorrhagic disease, which makes formula-fed infants resistant to VKA.
- Breast milk has low concentrations of vitamin K, making breastfed infants sensitive to VKA, which can be compensated for by feeding 30 to 60 ml of formula each day.

- VKAs are available only in tablet form in most countries, thus being unsuitable for newborns even if suspended in water.
- VKA requirements change rapidly across infancy because of rapidly changing physiological values of the vitamin K-dependent coagulation proteins, and changes in diet.
- There is little efficacy or safety information specific to VKA use in neonates.

However, for prosthetic valves, homozygous protein C deficiency and long-term therapy (beyond 3 to 6 months), VKA is probably superior to LMWH and can be managed in this age group by experienced teams and with adequate parental support.

Bleeding is the main complication of VKA therapy; however, in experienced hands the bleeding rates are reported to be less than 0.5% per patient year.

Approximately 30% of teenage girls on VKA develop menorrhagia, and proactive management of menstrual bleeding (often involving gynecological services) and attention to iron status is critical. A high proportion of teenagers who start VKA during their teenage years develop clinical depression or anxiety related to the psychosocial challenges involved in lifestyle restrictions. Proactive psychological support of these patients is important. Nonhemorrhagic complications of VKA, such as tracheal calcification or hair loss, have been described on rare occasions in young children. Reduced bone density in children on warfarin for greater than 1 year has been reported in a number of studies and many programs routinely monitor bone density in all children on long-term VKA.

Patient and family education protocols are major factors in reducing bleeding events in children on VKA therapy.

Direct oral anticoagulants

Several DOACs have been approved for a variety of indications in recent years. Most of them are small molecule inhibitors of coagulation factor Xa (anti-Xa drugs) or thrombin (anti-IIa drugs). They share several desirable attributes: (i) rapid onset of action; (ii) lack of need for routine monitoring of anticoagulant effect in most patients; (iii) relatively few clinically important interactions with medications; (iv) no dietary restrictions; and (v) short half-lives that simplify perioperative anticoagulation management. On the other hand, the dependence of some of these drugs on renal clearance limits their use in some patients. Four oral anti-Xa and 1 oral direct thrombin inhibitor are approved for various indications in the United States. Because the approved indications are expanding relatively rapidly as clinical trial data become available and

are being reviewed by the FDA, the reader is encouraged to obtain up-to-date approval status information when reading this section of this chapter. The names, molecular targets, and other pharmacologic properties of the 5 new oral anticoagulants furthest along in development are listed in Table 9-15, and include dabigatran, rivaroxaban, apixaban, edoxaban, and recently betrixaban.

No DOAC has completed trials in children yet, and currently they should not be used in children outside formal clinical trials.

Management issues

Several issues are important in management of patients who are being treated with DOACs.

First, although routine monitoring of the anticoagulant effect of these drugs is not necessary, measurement of their anticoagulant effect is helpful in selected clinical situations. For example, laboratory measurement of anticoagulant effect may be helpful for a bleeding patient, a patient in whom treatment failure is suspected, or a patient for whom the risks and benefits of urgent surgery are being considered. Data on expected therapeutic plasma drug levels determined by clinical bleeding and clotting events and the performance of the various coagulation tests have been published elsewhere. The ideal test for dabigatran is the dilute thrombin time or an ecarin-based assay. For FXa inhibitors (apixaban, rivaroxaban, edoxaban, and betrixaban), an anti-Xa activity—calibrated to the drug being measured—is preferred over other options. Many, but not all, aPTT assays are prolonged by clinically relevant concentrations of dabigatran. The same is true of the PT for rivaroxaban. For both medication-test combinations, the clinician should be aware of the sensitivity of his or her own laboratory's assays before interpreting the results.

Second, a growing body of evidence suggests that for patients whose thrombosis risk is low or moderate, DOACs can be interrupted for brief (<5 days) periods with a very low risk of thrombotic complications. The duration of preprocedural interruption is typically 24 to 72 hours but depends on renal function and the risk of bleeding inherent to the planned surgery.

Although both major intracranial bleeding and fatal bleeding occur less frequently with DOACs than with warfarin, major bleeding in patients taking these drugs can occur. A specific antidote, idarucizumab, has recently become available to immediately reverse the anticoagulant effect of dabigatran. For the direct factor Xa inhibitors, antidotes (andexanet alpha) are being developed. Despite the lack of antidotes for DOACs at the time of the randomized phase 3 trials, there is no evidence from either the pooled analyses of these trials or postapproval registry

Table 9-15 Direct oral anticoagulants: selected pharmacologic properties and approval status

Generic name	Apixaban	Dabigatran	Edoxaban	Rivaroxaban	Betrixaban
Brand name	Eliquis	Pradaxa	Lixiana, Savaysa	Xarelto	Bevyxxa
Target	FXa	FIa	FXa	FXa	FXa
T _{max} (h)	1–3	1.25–3	1–2	2–4	3–4
Half-life (h) in patients with normal renal function	8–15	12–14	8–10	9–13	19–27
Effect of hepatic impairment	Mild to moderate hepatic insufficiency (Child-Pugh A or B): no evidence of a consistent change in exposure	Moderate hepatic insufficiency (Child-Pugh B): no evidence of a consistent change in exposure	Moderate hepatic insufficiency (Child-Pugh B): no evidence of a consistent change in exposure	Moderate hepatic impairment (Child-Pugh B): increased mean exposure by 2.3-fold	Not evaluated
Renal excretion (%)	25	80	35–40	66	11
Effect of renal impairment	CrCL 30–50: 1.29-fold greater exposure	CrCL 30–50: 2.7-fold greater exposure	Not reported	CrCL 30–49: 1.5-fold greater exposure	60 to 90: 1.89 fold
	CrCL 15–29: 1.44-fold greater exposure	CrCL 10–30: 6-fold greater exposure (2-fold increase in the plasma half-life)		CrCL 15–29: 1.6-fold greater exposure	30 to 60 2.27 fold and 15 to 30 2.63 fold
Dosing frequency	Twice daily	Twice daily	Once daily	Once daily [†]	Once daily
Drug interactions	P-gp, CYP3A4	P-gp	P-gp	P-gp, CYP3A4	P-gp
Approval status as of February 2018 (United States)	Stroke prevention in AF; acute VTE treatment* and secondary VTE prevention; primary VTE prevention after total knee or hip replacement	Stroke prevention in AF; acute VTE treatment [‡] and secondary VTE prevention	Stroke prevention in AF; acute VTE treatment [‡] and secondary VTE prevention	Stroke prevention in AF; acute VTE treatment ^{†*} and secondary VTE prevention; primary VTE prevention after total knee or hip replacement	VTE prevention, moderate- and high-risk medically ill

AF, atrial fibrillation; CrCL, creatinine clearance (mL/min); VTE, venous thromboembolism.

*Apixaban is given 10 mg twice daily for the first 7 days in patients with acute VTE.

[†]Rivaroxaban is given 15 mg twice a day for the first 21 days in patients with acute VTE.

[‡]For dabigatran and edoxaban a 5-day "lead-in" with heparin or low-molecular-weight heparin is required in the treatment of acute VTE.

studies that major bleeding outcomes are worse in patients taking DOACs than in patients taking warfarin. Therapy with oral charcoal is appropriate in the patient who ingested the drug within 2 hours of presentation with major bleeding. FFP would not be expected to have any efficacy. Rivaroxaban, apixaban, edoxaban, and betrixaban cannot be removed with dialysis because they are highly bound to plasma proteins. Preclinical data from animal models, healthy volunteers, and ex vivo coagulation experiments suggest that PCCs may be of some benefit, but these interventions should be reserved for truly dire circumstances because they can cause thrombosis, and their benefit (if

any) in patients with DOAC-associated major bleeding is not established.

Very few patients with a diagnosis of APS, cancer, or warfarin failure were included in the VTE treatment trials of DOACs. However, as mentioned, data exist on the noninferiority of edoxaban and rivaroxaban, compared to LMWH, for the treatment of CAT. Because a study was stopped for increased thromboembolic events in the dabigatran arm compared to warfarin in a study of patients with mechanical prosthetic heart valves, DOACs should not be used to replace VKA treatment in a patient with a mechanical prosthetic heart valve.

Thrombolytic agents

A number of different thrombolytic (fibrinolytic) drugs are in clinical use, including streptokinase, urokinase, recombinant tPA, and tPA variants. All of them activate plasminogen to plasmin, which can then exert its thrombolytic effect on fibrin (Figure 9-4). In clinical practice, these drugs are used relatively rarely for venous thromboembolism because the associated risk of major bleeding is often not justified by the potential benefit. Streptokinase is derived from the culture of beta-hemolytic streptococci and urokinase is derived from the tissue culture of human neonatal kidney cells. Alteplase is a recombinant full-length, wild-type human tPA molecule of 527 amino acids. By deletion or substitution of functional domains or alteration of the molecules' carbohydrate composition, mutants of tPA have been produced. Reteplase is such a mutant tPA molecule, modified to be only 355 amino acids long. This leads to a longer half-life and better penetration into clots. Tenectapase is a recombinant full-length tPA molecule with 3 modifications, leading to increased binding of the molecule to thrombus-bound plasminogen compared with native tPA, as well as greater resistance to inactivation by its endogenous inhibitor (PAI-1).

No study has compared the efficacy, safety, or cost of different thrombolytic agents in children. However, tPA has become the agent of choice in pediatric patients. There is minimal experience with other thrombolytic agents in children, and little consensus in indications for thrombolysis, dose, mode of delivery, or duration of therapy, reflecting the lack of good quality studies. At this time, there is no evidence to suggest that there is an advantage of local over systemic thrombolytic therapy in children with thrombotic complications.

Success rates for thrombolysis in pediatric patients vary. Thrombolysis is usually used when there is limb- or life-threatening thrombosis of arterial or venous origin. In that context, while there are a number of relative contraindications, there are no absolute indications to thrombolysis in children and careful discussion of risk-benefit ratio should be had with parents prior to therapy.

Infants have a relative plasminogen deficiency compared to adults and common practice is to give FFP 10 ml/kg prior to tPa, in an effort to provide better plasminogen substrate for the tPa and to reduce bleeding through improved fibrinogen levels. Thrombolytics may not inhibit clot propagation, hence thrombin inhibition is required as adjunctive therapy. Concurrent low-dose UFH (10 U/kg/h) followed by therapeutic UFH is usually recommended.

The optimal dose of tPa is uncertain, but most protocols use 0.5 mg/kg/h for a maximum of 6 hours. Some centers recommend doses as low as 0.05 mg/kg/h. Reports

of accelerated tPA, especially in the setting of cardiac infarction associated with Kawasaki's disease, have been described.

Thrombolytic therapy has significant bleeding complications in children, with major bleeding reported in 10% to 30% of patients depending on patient selection. The intracerebral bleeding rate is probably less than 5% but may be increased in neonates. Thus, the bleeding risk from thrombolysis in children is at least an order of magnitude higher than the bleeding risk from anticoagulation alone. This risk needs to be weighed against the potential benefits of therapy in any child considered for thrombolysis. The bleeding rate may be related to duration of thrombolysis infusion and many centers recommend limiting the time of infusion to less than 6 hours. If further lysis is required, additional doses can be given over the next 24 hours.

Antiplatelet agents

Aspirin

Aspirin (acetylsalicylic acid) inhibits the enzyme cyclooxygenase-1 (COX-1), which is needed to form thromboxane A₂ in platelets. Thromboxane A₂ normally is released from platelet granules upon platelet adhesion and during platelet aggregation and serves as an agonist to activate, and thus recruit, other platelets to the platelet plug. Because platelets do not synthesize new cyclooxygenase and aspirin binds irreversibly to COX-1, full recovery of thromboxane production of the platelet pool after stopping aspirin takes approximately 10 days (ie, the platelets' life span). Recovery of platelet aggregation is quicker, however, occurring within 4 days of stopping aspirin, because thromboxane from newly synthesized platelets can activate aspirin-affected platelets. Complete inactivation of platelet COX-1 typically is achieved with a daily dose of 160 mg of aspirin. When used as an antithrombotic drug, aspirin is maximally effective at doses between 50 and 325 mg per day. In most clinical situations, higher doses increase the likelihood of toxicity (gastric ulceration and bleeding) but have not been consistently shown to improve efficacy.

In children, aspirin doses (when being used for antiplatelet therapy) vary from 1 to 5 mg/kg/d, with maximal dose of 100 mg/d. Gastrointestinal toxicity appears less in younger children. Reye's syndrome was associated with doses above 40 mg/kg/d, so higher doses should be avoided.

Phosphodiesterase inhibitors

Dipyridamole

Dipyridamole leads to an increase in intraplatelet cyclic adenosine monophosphate (cAMP) levels, which inhibits platelet aggregation to several agonists. By itself, however, dipyridamole has little or no effect as an antithrombotic drug. Its platelet aggregation inhibitory effect is revers-

ible. The combination of aspirin 25 mg and dipyridamole 200 mg in a sustained-release formulation is available as Aggrenox. Dipyridamole also has vasodilatory effects, and therefore should be used with caution in patients with severe coronary artery disease in whom episodes of angina may increase due to the steal phenomenon. Aggrenox has its major indication in secondary stroke prevention.

Cilostazol

Cilostazol is a selective inhibitor of the phosphodiesterase-3 isoenzyme and leads to inhibition of agonist-induced platelet aggregation, granule release, and thromboxane A₂ production. It also has vasodilator effects and should not be used in patients with congestive heart failure. Cilostazol has its major indication in disabling claudication, particularly when revascularization cannot be performed.

Pentoxyphylline

Pentoxyphylline is a phosphodiesterase inhibitor that has been shown to have some beneficial effects in ischemic disease states. Its inhibitory action on phosphodiesterase in erythrocytes leads to increased cAMP levels and improved erythrocyte flexibility, and reduction of blood viscosity may be the result of decreased plasma fibrinogen concentrations and inhibition of red blood cell and platelet aggregation. The major indication for pentoxyphylline is peripheral arterial disease with claudication.

Adenosine diphosphate receptor antagonists

Clopidogrel and ticlopidine

Clopidogrel and ticlopidine inhibit the adenosine diphosphate (ADP) receptor P2Y₁₂ by irreversibly altering its structure. Both drugs are closely related, but clopidogrel has a more favorable side-effect profile with less frequent thrombocytopenia and leukopenia, and therefore has replaced ticlopidine in clinical use. Because maximal inhibition of platelet aggregation is not seen until days 8 to 11 after starting therapy, loading doses of these drugs often are given to achieve a more rapid onset of action. Inhibition of platelet aggregation persists for the life span of the platelet. In all indications, clopidogrel appears to be as effective as aspirin, except in peripheral arterial disease, where it has been shown to be slightly more effective for the prevention of ischemic events. Clopidogrel is a prodrug, activated in the liver by cytochrome p450 enzymes, including CYP2C19. Genetic polymorphisms in CYP2C19 lead to decreased clopidogrel metabolism, and thus to a decreased antiplatelet effect. It is unclear, however, whether switching patients who are poor clopidogrel metabolizers to a different antiplatelet agent is clinically beneficial.

Clopidogrel is frequently used in children, particularly in cardiac surgical patients. The PICLO trial focused on children with congenital heart disease and determined that a dose of 0.20 mg/kg/d in infants and young children achieved platelet inhibition levels similar to those in adults taking the standard adult dose of 75 mg/d, although the validity of the study outcomes is likely significantly flawed. The CLARINET study reported a primary efficacy endpoint of a composite of death or heart transplantation, shunt thrombosis, or performance of a cardiac procedure due to an event considered to be thrombotic in nature within 30 days following modified Blalock-Taussig shunt. The outcomes were comparable for the clopidogrel (plus aspirin) group and the placebo (aspirin) group, with similar rates of overall bleeding and severe bleeding.

Prasugrel, ticagrelor, and cangrelor

Prasugrel, ticagrelor, and cangrelor also are inhibitors of the platelet P2Y₁₂ receptor. In comparison with clopidogrel, they are more rapid in onset, lead to less variable platelet response, and more complete inhibition of platelet function. Prasugrel-mediated inhibition is irreversible, while the P2Y₁₂ inhibition induced by ticagrelor is reversible.

Glycoprotein IIb/IIIa receptor antagonists

The platelet glycoprotein IIb/IIIa (GPIIb/IIIa) receptors are the sites where fibrinogen binds during platelet aggregation, resulting in cross-linking of platelets and platelet plug formation. Several inhibitors of this receptor have been developed and are in clinical use.

Abciximab

Abciximab is the Fab fragment of a chimeric human-murine monoclonal antibody against the IIb/IIIa receptor. The drug is given as a bolus, followed by a continuous infusion for 12 hours or longer. The unbound drug is cleared from the circulation with a half-life of about 30 minutes. Drug bound to the IIb/IIIa receptor inhibits platelet aggregation for 18 to 24 hours, measured in vitro, but the bound drug is demonstrable in the circulation for up to 10 days. Ex vivo platelet clumping in ethylenediaminetetraacetic acid-containing blood tubes can be seen in patients treated with the drug, leading to pseudothrombocytopenia when platelets are enumerated by an automatic blood cell counter. This phenomenon is clinically irrelevant and does not require discontinuation of the drug. True thrombocytopenia also occurs and, if severe enough, can require drug discontinuation.

Eptifibatide

Eptifibatide is a synthetic peptide inhibitor of the arginine-glycine-aspartic acid (so-called RGD) binding site of the

IIb/IIIa receptor. It mimics the geometric and charge characteristics of the RGD sequence of fibrinogen, thus occupying the IIb/IIIa receptor and preventing binding of fibrinogen, and thereby preventing platelet aggregation. It is given as a bolus, followed by a continuous infusion for up to 3 days. The platelet aggregation inhibitory effect lasts for 6 to 12 hours after cessation of infusion.

Tirofiban

Tirofiban is a nonpeptide (peptidomimetic), small-molecule inhibitor of the IIb/IIIa receptor, which also binds to the RGD receptor site, similar to eptifibatide.

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10

Bleeding disorders

RITEN KUMAR AND CHRISTINE L. KEMPTON

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The online version of this chapter contains an educational multimedia component on coagulation of blood.

Overview of hemostasis

Hemostasis is the process through which bleeding is controlled at a site of damaged vascular endothelium and represents a dynamic interplay between the subendothelium, endothelium, circulating cells, and plasma proteins. The hemostatic process often is divided into 3 phases: vascular, platelet, and plasma. Although it is helpful to divide coagulation into these phases for didactic purposes, *in vivo* they are intimately linked and occur in a continuum. The *vascular phase* is mediated by the release of locally active vasoactive agents that result in vasoconstriction at the site of injury and reduced blood flow. Vascular injury exposes the underlying subendothelium and procoagulant proteins, including von Willebrand factor (VWF), collagen, and tissue factor (TF), which then come into contact with blood. During the *platelet phase*, platelets bind to VWF incorporated into the subendothelial matrix through their expression of glycoprotein 1b-alpha (GP1b-alpha). Platelets bound to VWF form a layer across the exposed subendothelium, a process termed *platelet adhesion*, and subsequently are *activated* via receptors, such as the collagen receptors, integrin $\alpha_2\beta_1$, and glycoprotein (GPVI), resulting in calcium mobilization, granule release, activation of the fibrinogen receptor, integrin $\alpha_{IIb}\beta_3$, and subsequent *platelet aggregation* (Figure 10-1). For a detailed discussion of platelet function, please see Chapter 11.

The *plasma phase* of coagulation can be further subdivided into initiation, priming, and propagation (Figure 10-2; see video in online edition). Initiation begins when vascular injury also leads to exposure of TF in the subendothelium and on damaged endothelial cells. TF binds to the small amounts of circulating activated factor VII (FVIIa), resulting in formation of the TF:FVIIa complex, also known as the extrinsic tenase complex; this complex binds to and activates factor X (FX) to activated FX (FXa). FXa forms a complex with activated factor V (FVa), released from collagen-bound platelets, to convert a small amount of prothrombin to thrombin. The small amount of thrombin generated at this stage is able to initiate coagulation and generate an amplification loop by cleaving factor (F) VIII from VWF, activating FVIII, FXI, and platelets, which result in exposure of membrane phospholipids and further release of partially activated FV. At the end of the initiation and priming phases, the platelet is primed with an exposed phospholipid surface with bound activated cofactors (FVa and FVIIIa). During the propagation phase, activated factor IX (FIXa), gener-

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Off-label drug use: Dr. Kumar and Dr. Kempton: rFVIIa for management of bleeding in hemophilia at doses and regimens that are not approved and for other off-label indications; and prothrombin complex concentrates for treatment of factor II and X deficiency.

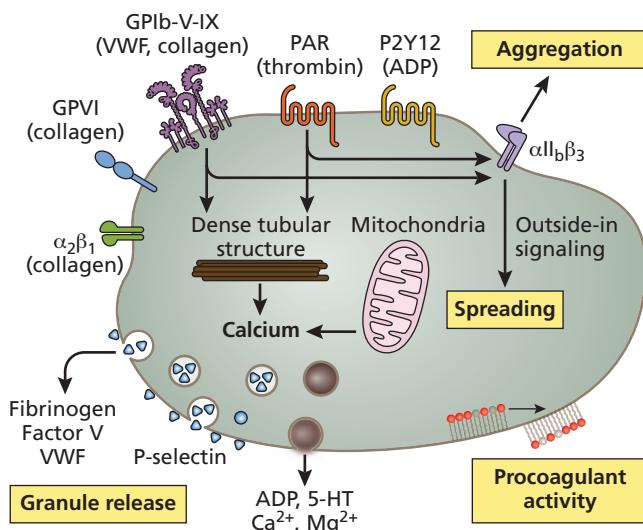


Figure 10-1 Platelet activation. Platelets can undergo activation through stimulation by soluble agonists, such as thrombin, or by contact (adherence) to the subendothelial matrix. This simplified cartoon shows several platelet components, including receptors and granules as well as the pathways of activation and the effect on platelet responses, such as aggregation, spreading, granule release, and procoagulant activity.

ated either by the action of FXIa on the platelet surface or TF-VIIa complex on the TF-bearing cell, binds to its cofactor FVIIIa to form the potent intrinsic tenase complex. FX is then bound and cleaved by the tenase complex (FIXa:FVIIIa), leading to large amounts of FXa, which in association with its cofactor, FVa, forms the prothrombinase complex on the activated platelet surface. The prothrombinase complex (FXa:FXa) then binds and cleaves prothrombin leading to an ultimate burst of thrombin sufficient to convert fibrinogen to fibrin (Figure 10-3) and result in subsequent clot formation. The formed clot is stabilized by the thrombin-mediated activation of factor XIII (FXIII), which acts to cross-link fibrin, and thrombin-activatable fibrinolysis inhibitor (TAFI), which acts to remove lysine residues from the fibrin clot, thereby limiting plasmin binding. Ultimately, the clot undergoes fibrinolysis, resulting in the restoration of normal blood vessel architecture. The fibrinolytic process is initiated by the release of tissue plasminogen activator (tPA) near the site of injury. tPA converts plasminogen to plasmin, which (via interactions with lysine and arginine residues on fibrin) cleaves the fibrin into dissolvable fragments.

Both the hemostatic and fibrinolytic processes are regulated by inhibitors that limit coagulation at the site of injury and quench the reactions, thereby preventing systemic activation and pathologic propagation of coagulation. The hemostatic system has 3 main inhibitory pathways:

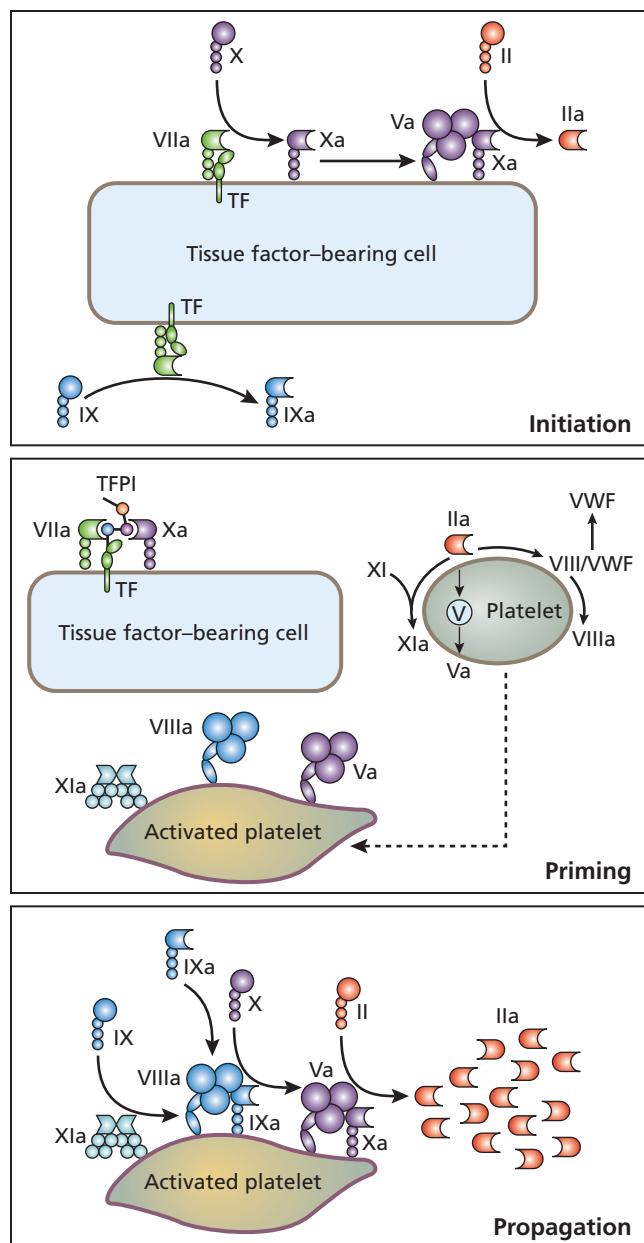


Figure 10-2 Thrombin generation occurs on 2 distinct cellular surfaces. The first is the tissue factor (TF)-bearing cell at the site of vascular injury. *Initiation* of coagulation occurs on the TF-bearing cell through generation of a small amount of thrombin that then goes on to *prime* the system by activating platelets, releasing FVIII from von Willebrand factor (VWF) and activating it, and activating factor XI. At the end of the priming step, the activated platelet with bound FXIa and cofactors FVa and FVIIIa are ready to form essential complexes, tenase (FVIIIa:FIXa) and prothrombinase (FVa:FXa) and through an amplification loop can *propagate* thrombin generation, forming a burst of thrombin capable to form a hemostatic fibrin clot.

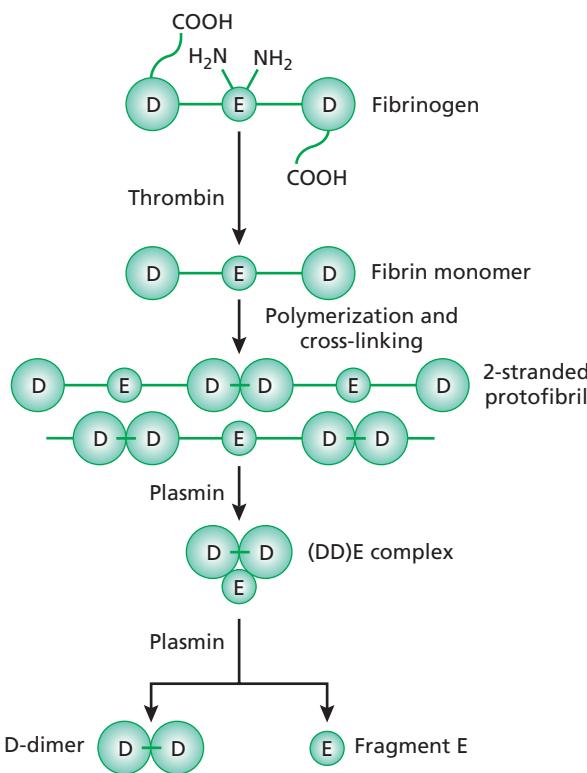


Figure 10-3 Fibrin formation and degradation. Fibrinogen has a trinocular structure with a central E and 2 D domains. Thrombin cleaves fibrinopeptides A and B (not depicted), located in the E domain. The resultant fibrin monomers polymerize nonenzymatically forming fibrin polymers. Factor XIIIa cross-links the D domains of nearby fibrin monomers. Plasmin degrades cross-linked fibrin, thereby generating (DD)E complexes composed of an E fragment noncovalently bound to D-dimer. With further plasmin attack, the (DD) E complex is degraded into fragment E and D-dimer.

antithrombin (AT), the protein C:protein S complex, and tissue factor pathway inhibitor (TFPI). AT released at the margins of endothelial injury binds in a 1:1 complex with thrombin, inactivating thrombin not bound by the developing clot. AT also rapidly inactivates FXa; thus, any excess FXa generated by the TF:FVIIa complex during initiation is inactivated and unable to migrate to the activated platelet surface. Excess free thrombin at the clot margins binds to thrombomodulin, a receptor expressed on the surface of intact endothelial cells that when complexed with thrombin activates protein C. Activated protein C complexes with its cofactor protein S and inactivates FVa and FVIIIa. TFPI is a protein produced by endothelial cells that inhibits the TF:FVIIa complex and FXa. Binding to FXa is required for the inhibitory effect on TF:FVIIa. This negative feedback results in reduced subsequent thrombin generation and quenching of fibrin generation. The action of both AT and TFPI inhibits FXa during

the initiation phase, leading to dependence on platelet-surface FXa generation during the propagation phase for adequate hemostasis. The fibrinolytic system also includes 2 inhibitors, principally plasminogen activator inhibitor-1 (PAI-1) and α_2 -antiplasmin (α_2 AP), which inhibit tPA and plasmin, respectively.

This chapter is devoted to a discussion of the pathophysiology, clinical presentation, diagnosis, prognosis, and treatment of hemostatic abnormalities, hereafter referred to as bleeding disorders. The first section reviews the approach to a patient with excessive bleeding, followed by a discussion of the specific disorders.

KEY POINTS

- Hemostasis is a complex and highly regulated process involving the subendothelium, endothelial cells, circulating cells, and plasma proteins that include both positive and negative feedback mechanisms.
- The generation of thrombin is dependent on specific protein complexes occurring on cellular surfaces: TF:FVIIa complex at the site of injury and FIXa:FVIIIa (tenase complex) and FXa:FVa (prothrombinase complex) on the activated platelet surface.

Approach to the patient with excessive bleeding

Excessive bleeding may occur in both male and female patients of all ages and ethnicities. Symptoms can begin as early as the immediate newborn period (uncommonly even *in utero*) or anytime thereafter. The bleeding symptoms experienced are related in large part to the specific factor and level of deficiency. Bleeding can be spontaneous; that is, without an identified trigger, or may occur after a hemostatic challenge, such as delivery, injury, trauma, surgery, or the onset of menstruation. Furthermore, bleeding symptoms may be confined to specific anatomic sites or may occur in multiple sites. Finally, bleeding symptoms may be present in multiple family members or may occur in the absence of a family history. All of this information is important to arrive at a correct diagnosis rapidly and with minimal, yet correctly sequenced, laboratory testing. Thus, a detailed patient and family history is a vital component of the approach to each patient with a potential bleeding disorder.

Importance of medical history

Obtaining a detailed patient and family history is crucial regardless of prior laboratory testing. The history includes a detailed discussion of specific bleeding and clinical symptoms. Information regarding bleeding symptoms

should include location, frequency, and pattern; as well as duration both in terms of age of onset and time required for cessation. The location may suggest the part of the hemostatic system affected; patients with disorders of primary hemostasis (platelets and VWF) often experience mucocutaneous bleeding, including easy bruising, epistaxis, gingival hemorrhage with dental hygiene, heavy menstrual bleeding, and postpartum hemorrhage in women of childbearing age; whereas patients with disorders of secondary hemostasis (coagulation factor deficiencies) may experience deep-tissue bleeding, including the joints, muscles, and central nervous system. The bleeding pattern and duration of each episode, particularly for mucus membrane bleeding, assist in the determination of the likelihood of the presence of an underlying bleeding disorder. The onset of symptoms can suggest the presence of a congenital vs acquired disorder. Although congenital conditions can present at any age, it is more likely that patients with a long history of symptoms or symptoms that begin in childhood have a congenital condition; whereas patients whose onset occurs at an older age are more likely to have an acquired condition. Congenital clotting factor deficiencies that do not present until later in life do occur and include mild factor deficiencies and coagulation factor deficiencies associated with variable bleeding patterns, most notably FXI deficiency. Additional important information to be collected includes the current use of medications and herbal supplements, as these may affect the hemostatic system; the presence or absence of a family history of bleeding; a history of hemostatic challenges, including surgery, dental procedures, and trauma; and a menstrual history in females. The goal at the end of the history is to establish the likelihood of a bleeding disorder, as this guides the direction of the laboratory investigation.

Bleeding assessment tools

As discussed above, determining the presence and severity of bleeding symptoms is a key component in evaluating a patient with a suspected bleeding disorder. However, mild bleeding symptoms are routinely reported in the “healthy” population and differentiating “pathological” from “normal” bleeding symptoms may be difficult. To meet these challenges, multiple attempts have been made to develop and validate objective frameworks for the evaluation of bleeding symptoms. Bleeding assessment tools (BATs) are standardized instruments that quantify the presence and severity of bleeding symptoms to generate a single score. The Vicenza score and its successor, the Molecular and Clinical Marker for the Diagnosis and Treatment of Type 1 von Willebrand disease (VWD) (MCDM-1VWD), were the first to be developed and studied for patients with VWD. A pediatric version of the MCDM-1VWD

was subsequently developed and included pediatric-specific questions on umbilical stump bleeding, postcircumcision bleeding, and cephalohematoma. In 2010, the International Society on Thrombosis and Haemostasis (ISTH) endorsed a consensus-based questionnaire and grading instrument (ISTH-BAT), which provides a summated score based on 14 bleeding symptoms.

These instruments have demonstrated the ability to distinguish patients with VWD from healthy subjects, as well as predict future bleeding risk. While extremely useful as research tools, the applicability of BATs to clinical practice requires further investigation. Given their high negative predictive value, their greatest utility would likely be in identifying patients where laboratory testing is not necessary.

Screening tests

The laboratory evaluation for bleeding includes performance of initial screening tests. The most common screening tests utilized include platelet count, prothrombin time (PT), and activated partial thromboplastin time (aPTT). When the PT or aPTT is prolonged, mixing studies are required via a 1:1 mix of patient plasma with known normal standard plasma. Test correction in the mixing study indicates a deficiency state; whereas lack of correction indicates an inhibitor, either one directed against a specific factor (eg, FVIII in acquired hemophilia) or a global inhibitor as best exemplified by a lupus anticoagulant. Inhibitors directed against FVIII in acquired hemophilia are typically time- and temperature-dependent; therefore, incubated mixing studies should be performed (incubating the patient plasma with normal standard plasma at 37° for 1 to 2 hours). Specific factor analyses are performed after mixing studies reveal a correction of prolonged coagulation screening test(s) indicative of a deficiency state, or in the face of normal screening tests with a positive history. Screening tests are not sensitive and do not evaluate all abnormalities associated with bleeding—including VWF, FXIII, PAI-1, and α_2 AP deficiencies—and may be insensitive to mild FVIII and FIX deficiencies; therefore, a patient history suggestive of a bleeding disorder may warrant testing for such deficiencies, including rare abnormalities regardless of screening test results.

Screening tests also are utilized to identify individuals with a high likelihood of VWD or platelet disorders. Bleeding time, once widely used, has become obsolete because of the lack of sensitivity and specificity. The PFA-100 (platelet function analyzer) has been proposed as having a role in screening individuals with suspected platelet dysfunction or VWD. Initial studies demonstrated the efficacy of the PFA-100 in the evaluation of patients with known severe platelet disorders or VWD. The PFA-100 induces high shear stress and simulates primary hemostasis by flowing whole blood

through an aperture with a membrane coated with collagen and either adenosine diphosphate (ADP) or epinephrine. Platelets adhere to the collagen-coated surface and aggregate, forming a platelet plug that enlarges until it occludes the aperture, causing cessation of blood flow. The time to cessation of flow is recorded as closure time (CT). The sensitivity and specificity of the CT of the PFA-100 were reported as 90% for severe platelet dysfunction or VWD, with VWF plasma levels below 25%. The utility of the PFA-100 as a screening tool, however, has been challenged based on the reported low sensitivity (24% to 41%) of the device in individuals with mild platelet secretion defect, mild VWD, or platelet storage pool disorders. Additionally, a significant limitation of the PFA-100 is the fact that platelet count and hemoglobin levels affect the CT. The CT is abnormal if the platelet count is less than 100,000/ μ L and hemoglobin is <10 g/dL.

It is likely that by the time patients are referred to a hematologist that some, if not all, of the previously mentioned tests may have been performed. Screening tests are sensitive to specimen handling, may vary in reliability based on laboratory, and may be influenced by medications. Repeating these laboratory tests often is required; if possible, it is best to discontinue medications known to affect their results. Therefore, although screening tests are used widely to identify hemostatic abnormalities associated with bleeding, they are not perfect. The clinical suspicion for a bleeding disorder is critical to determine extent of the laboratory investigation.

KEY POINTS

- Patients with bleeding disorders occasionally present for evaluation before symptom onset, especially in the presence of a known family history or abnormal screening laboratory tests.
- Patients with bleeding disorders can present at any age with bleeding in a variety of sites. The more severe disorders tend to present earlier in life and with bleeding symptoms that often are spontaneous or in such areas as the joints, muscles, or central nervous system.
- The approach to patients with a potential bleeding disorder requires a detailed personal and family history and involves the use of screening laboratory tests, mixing studies when results are abnormal, and subsequent further specific coagulation factor testing.
- Some patients with a history or physical examination indicative of a bleeding disorder may have a normal laboratory evaluation. A study by Quiroga et al. showed the diagnostic efficacy of laboratory testing in patients with hereditary mucocutaneous bleeding is approximately 40%.

Disorders of primary hemostasis

Platelet function disorders

Pathophysiology

Platelets play a key role in primary hemostasis, both by constituting the cellular structure for the primary hemostatic plug and providing a phospholipid surface upon which plasma coagulation proteins bind and form complexes. Low platelets or impaired platelet function may result in bleeding; thrombocytopenic and platelet function defects are reviewed in detail in Chapter 11. Abnormalities in platelet function can occur in any of the multitude of processes required for normal platelet function, including defects in receptor number or function, signaling, and granule content and secretion. An overview of platelet pathophysiology is important to the understanding of described platelet function defects.

A simplified cartoon with the platelet major receptors and activation responses is shown in Figure 10-1. Platelet activation is the result of multiple signaling pathways that culminate in activation of the fibrinogen receptor integrin $\alpha_{IIb}\beta_3$, an integrin that normally exists in a resting (low-affinity) state but that transforms into an activated (high-affinity) state when stimulated by the appropriate signal transduction cascade. Activated $\alpha_{IIb}\beta_3$ then mediates platelet aggregation and promotes stable thrombus formation. This activation occurs following vascular injury when subendothelial collagen engages $\alpha_2\beta_1$ and GPVI receptors, and turbulent shear stress promotes VWF binding to GP1b-IX-V. A process known as inside-out signaling follows this platelet surface receptor stimulation, leading to activation of $\alpha_{IIb}\beta_3$ and resulting in affinity modulation during thrombus initiation. This conformational change allows engagement of fibrinogen by multiple $\alpha_{IIb}\beta_3$ integrins, resulting in platelet aggregation. Subsequently, outside-in signaling is initiated when ligand-occupied $\alpha_{IIb}\beta_3$ integrins cluster during aggregation by binding fibrinogen, fibrin, or VWF; and trigger signals that stabilize the aggregate leading to activation responses—including granule release, platelet spreading, and clot retraction. During this multistep process, platelets also become activated through binding of agonists, such as ADP or thrombin, and secrete granular contents that enhance vasoconstriction and further platelet aggregation. Finally, the platelet membrane exposes negatively charged phospholipids, the surface upon which the plasma clotting factors bind and form the fibrin meshwork.

Etiology

Although this section briefly encompasses some of the most well-described defects, a full review of platelet func-

tion defects is included in Chapter 11, and a number of excellent review articles addressing this topic are available.

Defects at any stage of the platelet activation process can result in platelet dysfunction and subsequent bleeding. For example, absence or functional defects in GP1b-alpha results in Bernard-Soulier syndrome; whereas a gain-of-function mutation in the same receptor is associated with excess binding of VWF, resulting in platelet-type VWD, a rare bleeding disorder. Defects in the production, storage, and secretion of vasoactive and hemostatic molecules result in excessive bleeding. Such disorders are exemplified by the storage pool defect, which is associated with reduced secretion of ADP, and the gray platelet syndrome, a defect in α -granule formation. A defect in or absence of $\alpha_{IIb}\beta_3$ results in Glanzmann thrombasthenia, the most severe platelet function defect. Most platelet function defects are diagnosed via platelet aggregation. Identification of the causative defect or its presence in multiple family members implies a genetic abnormality.

Acquired platelet defects are most commonly the result of medications or herbal supplements, chronic medical conditions such as uremia, or medical interventions such as cardiopulmonary bypass. The list of medications associated with platelet dysfunction is large. The most commonly used medications that result in platelet dysfunction, many of which are available over the counter, include aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), antihistamines, guaifenesin, certain anti-convulsants (valproic acid in particular), antibiotics, and antidepressants (including, most commonly, selective serotonin reuptake inhibitors). Commonly used supplements, such as garlic, ginger, omega-3 fatty acids, vitamin E, and ginkgo biloba, also have been reported to affect platelet function. Thus, when a medical history is obtained, it is imperative to ask not only about prescribed medications but also over-the-counter and herbal supplements. Most of these medications and supplements do not lead to a clinically apparent bleeding disorder, but instead they often exacerbate clinical bleeding associated with a mild disorder or confound results of platelet function tests. Therefore, knowledge of all medications and supplements is critical to interpreting laboratory tests. It is also important to mention that severe anemia can exacerbate bleeding likely due to the impaired rheological effect of red cells that otherwise push platelets onto the damaged blood vessel wall.

Clinical presentation

Patients with platelet function disorders present with similar symptoms, regardless of the specific defect. The severity of symptoms is dictated by the specific condition and clinical situation. Patients with platelet function de-

ficts exhibit mucocutaneous bleeding similar to patients with VWD. Severe hemorrhage can occur in patients with profound thrombocytopenia or Glanzmann thrombasthenia. Patients may present to the hematologist as a result of abnormal bleeding, a known family history of bleeding either with or without a personal bleeding history, or an abnormal screening test (such as the PFA-100) obtained before a planned procedure.

Diagnosis

The diagnosis of platelet disorders is covered in Chapter 11. Briefly, the platelet count must be determined and the smear reviewed; platelet aggregation assays are abnormal in the setting of significant thrombocytopenia (ie, $<100 \times 10^9$), and the PFA-100 is abnormal with significant thrombocytopenia or anemia. Thus, a complete blood count (CBC) should be performed before obtaining platelet-specific studies. The 2 commonly available tests to screen for platelet function disorders both have limitations. The original screening test was bleeding time; as previously stated, bleeding time has fallen out of favor because of its limitations, particularly its inability to predict clinical bleeding.

PFA-100

The PFA-100 is a widely available laboratory test that may be abnormal in some congenital and acquired platelet function disorders. Patients with severe platelet function defects, such as Bernard-Soulier syndrome and Glanzmann thrombasthenia, also have abnormal results. The CT is often abnormal in patients on aspirin, NSAIDs, and clopidogrel. The effects of other medications known to affect platelet function, such as valproic acid, are not clear. The utility of the CT is limited by insufficient sensitivity, such that it rarely obviates the need for further testing, and its inability to distinguish between the 2 most common bleeding disorders (ie, platelet function defects and VWD). The CT may be abnormal in mild disorders, such as common platelet secretion defects; however, its sensitivity for these disorders is insufficient to rule out such defects in the face of a normal result.

Platelet aggregometry

The most specific assay of platelet function is platelet aggregation by light transmission aggregometry. This assay uses platelet-rich plasma (PRP) and evaluates platelet aggregation via light transmission after the addition of a variety of agonists, such as ADP, epinephrine, ristocetin, arachidonic acid, collagen, and thrombin-related activation peptide. Recent recommendations by the ISTH Subcommittee on Platelet Physiology expanded the use of agonists to the thromboxane A2 mimetic U46619. Patients with a variety of both severe and mild platelet function disorders

exhibit abnormal platelet aggregation profiles and furthermore, the spectrum of abnormalities can be diagnostic of specific disorders. For example, if results demonstrate absent aggregation to all agonists except ristocetin, the pattern is diagnostic of Glanzmann thrombasthenia, whereas normal aggregation to all agonists and absent response to ristocetin is consistent with Bernard-Soulier syndrome. In addition, a pattern of aggregation followed by disaggregation with ADP is consistent with secretion defects. Luminescence, commonly used in combination with platelet aggregation, provides a sensitive evaluation of adenosine triphosphate release from dense granules. Adenosine triphosphate released by the platelets provides energy for the added light-producing enzyme luciferase, and a light burst is recorded. In patients with a dense granule deficiency or platelet release defect, this burst is impaired. A more detailed discussion of platelet aggregation can be found in reviews of platelet function disorders.

As with the PFA-100 CT, several preanalytical variables may affect the results of the test. Many medications and supplements have been reported to affect platelet aggregation studies; therefore, the assay should be performed when patients are no longer receiving these medications or supplements for approximately 10 days. It is recommended that individuals to be tested refrain from consuming alcohol, caffeine, tobacco, or high fat-content meals several hours before the test. Ideally, platelet aggregation should be performed in fasting state and abstaining from flavonoids for several days. The assay can be performed in anemic and even thrombocytopenic patients (if one suspects a platelet function defect in addition to thrombocytopenia) because it is performed on PRP. For thrombocytopenic patients, the amount of blood required may be prohibitive, and consultation with the coagulation laboratory is recommended before ordering the assay in this circumstance. Although most laboratories in the United States use PRP for aggregometry studies, whole blood aggregometry is also available in some centers, with reported reliable results.

Flow cytometry

Flow cytometry may be employed to quantify levels of platelet surface receptors and can confirm the diagnosis of Bernard-Soulier syndrome and Glanzmann thrombasthenia. In some institutions, these assays are available and have become the method of choice for diagnosis but have not been standardized for widespread use.

Electron microscopy

Some platelet function defects lead to easily identifiable platelet ultrastructural changes visualized by electron microscopy. In particular, patients with a deficiency or abnormalities of dense bodies (δ -storage pool deficiency) or

α -granules (gray platelet syndrome and Paris-Trousseau syndrome) can be diagnosed by this method.

Finally, and because most of the genes responsible for these disorders have been identified, genetic testing is available for selected families and may guide future therapeutic strategies as well as provide information for genetic counseling. Additionally, it is important to remember that some platelet disorders may have systemic manifestations that should be explored, such as the presence of oculocutaneous albinism or pulmonary fibrosis in patients with Hermansky-Pudlak syndrome.

Treatment

Congenital platelet function defects may benefit from medical modalities for hemostatic control; although ultimately, platelet transfusions may be required if medications or local measures are ineffective. In acquired conditions, treatment or reversal of the underlying condition resolves the platelet dysfunction; however, this is not always possible. In such situations, the approach to management of bleeding is similar to that for congenital disorders.

Patients with mild mucocutaneous bleeding episodes may be managed with topical adjunctive measures such as compression, and gelatin sponge or gauze soaked in tranexamic acid (TXA) for superficial wounds, or nasal packing and topical thrombin gel for epistaxis. Moderate to major bleeding episodes may require medications that can enhance hemostasis, such as desmopressin, antifibrinolytic agents, estrogen, recombinant factor VIIa (rFVIIa), and platelet transfusion.

Desmopressin is a synthetic analogue of the antidiuretic hormone vasopressin and exerts its procoagulant effect by increasing the circulating levels of FVIII and VWF. While desmopressin can improve platelet function in congenital platelet disorders, uremia, and during cardiopulmonary bypass, the specific mechanism of action is not clear. Desmopressin may be administered intravenously, subcutaneously, or intranasally (Stimate; CSL Behring, King of Prussia, PA). The standard dose of desmopressin is 0.3 μ g/kg administered intravenously or subcutaneously, or 300 μ g administered intranasally. In some circumstances, it may be useful to perform a desmopressin challenge test before its clinical use. The challenge test entails assessment of platelet function before and approximately 90 minutes after administration; however, it is recognized that a poor correlation exists between the results of platelet function tests and clinical outcomes, and thus the value of this approach is uncertain. Desmopressin is a relatively safe agent, although its use can lead to vasomotor symptoms resulting in headache, tachycardia, and facial flushing, with rare reductions in blood pressure sufficient to result in clinical symptoms. Moreover, as an analog of an antidiuretic

hormone, desmopressin can result in water retention, hyponatremia, and (rarely) seizures. Although seizures rarely occur in adults and older children, the risks are increased in young children and those receiving intravenous fluids. Therefore, an experienced care provider should oversee its use. Additionally, patients should be instructed to limit their fluid intake for 24 hours after desmopressin use. Repeated use at short intervals should be limited because of the development of tachyphylaxis. Desmopressin should not be used in children under 2 years of age because of the high risk of hyponatremic seizures.

Antifibrinolytic agents (aminocaproic acid [EACA] and TXA) are commonly used adjunctive hemostatic therapies. These agents, which are lysine analogues, inhibit plasmin-mediated thrombolysis and exert their effect through clot stabilization and prevention of early dissolution. Thus, these agents may be effective in prevention of rebleeding, a common problem in individuals with bleeding disorders, especially in areas with increased fibrinolysis, such as the gastrointestinal tract. These agents may be administered intravenously, orally, or topically in amenable circumstances, and are used either therapeutically for bleeding or prophylactically as part of perioperative management. Treatment of mucosal bleeding commonly includes the use of antifibrinolytic agents in conjunction with desmopressin; this combination is also effective in bleeding from other sites—for example, in the management of heavy menstrual bleeding. Antifibrinolytic agents have been used widely for many years, have a documented safety profile, and are well tolerated in most patients. Commonly reported side effects include headache and abdominal discomfort; however, these symptoms do not preclude its continued use if ameliorated with other agents, such as acetaminophen. Antifibrinolytic agents should be used with caution in patients with a history of thrombosis or atherosclerosis and are contraindicated when hematuria is present because obstructive uropathy secondary to ureteral clots may develop.

Estrogens have documented effectiveness in the management of excessive menstrual bleeding. The mechanism of action is not well elucidated, although their use is associated with an increase in procoagulants, including VWF and FVIII, and a decrease in naturally occurring coagulation inhibitors, particularly protein S. Conjugated estrogens also are used for the management of severe heavy menstrual bleeding, with both the previously mentioned hemostatic effects and the additional local effect of reduced uterine blood flow. Estrogen in combination with progestins, as in oral contraceptive agents, is useful for home management of heavy menstrual bleeding in patients with bleeding disorders, including platelet function disorders and VWD. The positive effects of these agents are

likely similar to conjugated estrogens in conjunction with progestin-induced stabilization of the endometrial lining. The levonorgestrel intrauterine devices (IUDs) are also very effective for management of heavy menstrual bleeding. The risks associated with estrogens include thrombosis; thus, these agents should be avoided in patients with a personal history of thrombosis or who are deemed at high risk for thrombosis.

Although rFVIIa has been shown anecdotally to be effective for the management of severe bleeding in patients with platelet function defects, its value in this setting is not clearly defined. rFVIIa is costly and may be associated with adverse events, including thrombosis; therefore, its use should be supported by evidence of its efficacy and judicious utilization. rFVIIa is licensed in the European Union and in the United States for the management of bleeding in patients with Glanzmann thrombasthenia refractory to platelet transfusions. For severe bleeding, especially in patients with Bernard-Soulier syndrome and Glanzmann thrombasthenia, platelet transfusion should be administered to provide normally functioning platelets. The general risks associated with platelet transfusion, common to all patients, include the risk of transfusion reactions and potential transmission of infectious agents (see Chapter 11 for details on risks of platelet transfusions). A more important specific risk associated with Bernard-Soulier syndrome and Glanzmann thrombasthenia is alloimmunization because of the formation of antibodies against the absent receptor. Once antibodies develop, future platelet transfusions are likely to be ineffective and may be associated with unusual reactions. Thus, judicious use of platelet transfusions is imperative in these patients.

Education of patients and primary care providers is important so that bleeding episodes are either prevented or recognized early and managed locally. Lifestyle modifications are important and include: avoidance of collision and contact sports, routine dental care, use of medical-alert bracelets and avoidance of platelet-impairing medications (eg, aspirin, NSAIDs). Patients should be advised to report their condition to physicians before undergoing any invasive procedures so that appropriate prophylactic measures can be used.

Prognosis and outcomes

The majority of commonly encountered platelet function disorders are associated with mild intermittent bleeding episodes that do not significantly interfere with daily life. Disorders like Glanzmann thrombasthenia, however, can be associated with significant bleeding that profoundly affects quality of life. In some patients, bleeding is so severe that bone marrow transplantation has been undertaken to correct the defect by replacing the population of megakaryocytes.

This extreme approach is reserved only for the most severe patients in whom an unaffected human leukocyte antigen-compatible sibling is available.

Gaps in knowledge

The complexity of establishing a correct diagnosis cannot be underestimated as the first and most important step in the appropriate management of patients with platelet function disorders. Although current laboratory assays are helpful, patients may be left without a more specific diagnosis other than the broad category of a platelet function defect. The complexities of platelet structure and function make identification at a molecular or cellular level impractical or impossible in many patients outside of specialized research centers. Therefore, an important area for future research is the development of widely available laboratory assays with increased sensitivity and specificity that are able to unravel platelet function defects into better defined categories. Some promising approaches, such as the use of platelet proteomics and platelet adhesion assays under flow conditions, are being developed and improved. Although these assays presently are used only in a research setting, it is feasible that further work will allow development of clinically useful versions. In addition, the ongoing development of global hemostatic assays may allow for identification of a patient's defect despite their previous evaluations being poorly defined or unrevealing. At present, a number of assays are under evaluation; it is hoped that in the relatively near future, these may become a part of the armamentarium available in the coagulation laboratory.

KEY POINTS

- Platelet function disorders can be congenital or acquired and typically present with mucocutaneous bleeding symptoms.
- Screening tests for platelet disorders have limited value. The gold standard laboratory evaluation for platelet function disorder involves platelet aggregation studies.
- Glanzmann thrombasthenia is the most severe platelet function defect and has the potential to result in significant bleeding requiring blood transfusion. Platelet transfusions in this disorder are reserved for life-threatening bleeding because of the risk of developing alloantibodies that render further transfusions ineffective.
- Secretion defects are among the most common platelet function defects and typically cause mild to moderate mucocutaneous bleeding symptoms that are managed with desmopressin, antifibrinolytic agents, and hormonal therapy for heavy menstrual bleeding.

von Willebrand disease

Pathophysiology

VWD is the most common congenital bleeding disorder in humans, with an estimated prevalence of 1 in 1,000 individuals. The transmission of VWD is autosomal dominant for most types but rarely may be inherited in a recessive manner (for type 2N and type 3VWD).

VWD is caused by the quantitative deficiency (type 1 and type 3) or qualitative defect (type 2) of VWF, a large, multimeric glycoprotein produced both in megakaryocytes and endothelial cells. Therefore, 2 pools of VWF are available for normal hemostasis. Circulating VWF is released from stored VWF in Weibel-Palade bodies of endothelial cells, whereas platelet VWF is stored in α -granules and released only upon platelet activation. The main roles of VWF in hemostasis are to (i) promote platelet adhesion to the exposed subendothelium; (ii) promote platelet aggregation; and (iii) serve as a chaperone for FVIII in plasma, protecting it from proteolytic degradation by activated protein C. VWF undergoes significant posttranslational modification, including dimerization, glycosylation, and multimerization before being packed into storage granules (Weibel-Palade bodies or α -granules) after cleavage of the VWF propeptide (VWFpp). VWFpp is released in equimolar concentrations to the mature VWF molecule, and is therefore useful in measuring the rate of clearance of mature VWF.

When in circulation, the molecular weight of VWF ranges from 500 kDa (short VWF multimers) to 20,000 kDa (high-molecular-weight multimers [HMWM]). Molecular size is an important determinant of functional activity, as the high-molecular-weight VWF multimers are the most physiologically active. The molecular weight of VWF is controlled by the metalloprotease enzyme ADAMTS13 (adisintegrin and metalloprotease with thrombospondin 1 motif, member 13), which cleaves the VWF in the A2 domain. Recent data suggest that VWF clearance is led in part by macrophages in the liver and spleen.

Classification of VWD

VWD is categorized into quantitative or qualitative VWF defects. VWD type 1 and type 3 represent partial and absolute quantitative deficiencies of VWF respectively; VWD type 2 is characterized by a qualitative defect in the von Willebrand protein. The ISTH has further subdivided type 2 VWD into 4 subtypes based on the exact physiological defect: 2A, 2B, 2M and 2N. Following is a brief description of the different subtypes and the molecular mechanisms that define them. Figures 10-4 and 10-5 illustrate these mechanisms and how they lead to the current

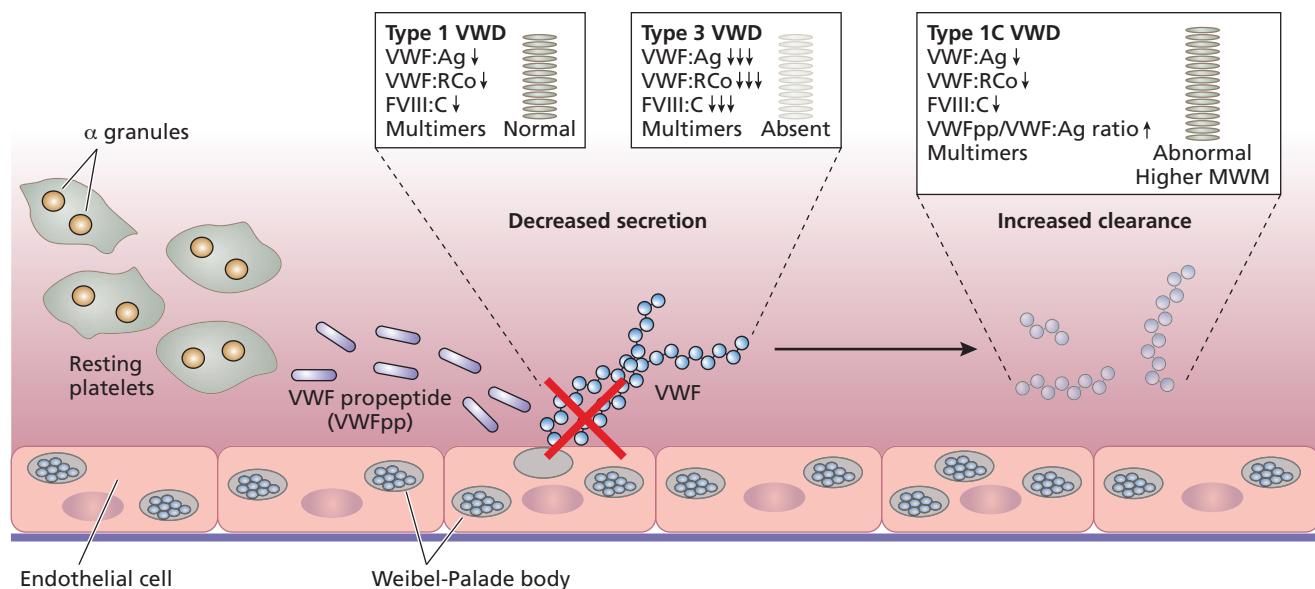


Figure 10-4 Mechanisms of disease for VWD types 1 and 3. Note that in boxes are shown the most common laboratory findings for these types. Redrawn from Branchford BR, Di Paola J, *Hematology Am Soc Hematol Educ Program*. 2012;2012:161–167.

classification. Table 10-1 describes the subtypes in more detail.

VWD type 1

VWD type 1 is defined by partial quantitative deficiency of VWF and bleeding symptoms. A family history of the disease or bleeding symptoms is usually present, though

its absence does not preclude the diagnosis. Patients with VWF levels <30 IU/dL usually have identifiable mutations in the VWF gene (*VWF*) and often report significant bleeding symptoms.

Patients with VWF levels between 30 and 50 IU/dL and a personal and family history of bleeding are often classified as having “low VWF.” In this subcohort, the

Figure 10-5 Mechanisms of disease for VWD type 2. Note that in boxes are shown the most common laboratory findings for the different subtypes. Redrawn from Branchford BR, Di Paola J, *Hematology Am Soc Hematol Educ Program*. 2012;2012:161–167.

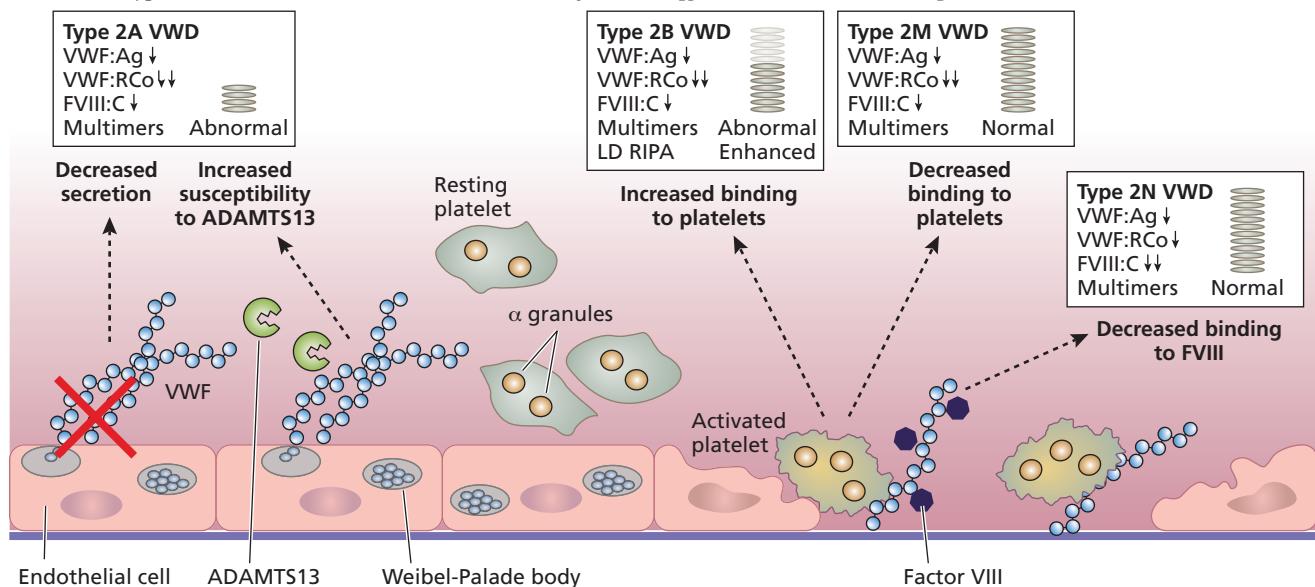


Table 10-1 Classification and diagnosis of von Willebrand disease (VWD)

Disease subtype	Description	VWF:RCo, IU/dL	VWF:Ag, IU/dL	VWF:RCo/VWF:Ag	FVIII level	Multimer pattern	RIPA
Type 1	Partial quantitative deficiency of VWF	<30*	<30*	>0.6	↓ or normal	Normal	Normal
Type 2A	Defect in multimerization or increased cleavage of multimers by ADAMTS13	<30†	30–200	<0.6	↓ or normal	Loss of high-molecular-weight multimers	↓
Type 2B	Increased affinity for platelet GP1b	<30†	30–200	<0.6	↓ or normal	Loss of high-molecular-weight multimers	↑
Type 2M	Decreased VWF mediated platelet adhesion	<30†	30–200	<0.6	↓ or normal	Normal	↓
Type 2N	Markedly decreased binding affinity for FVIII	30–200	30–200	>0.60	↓↓	Normal	Normal
Type 3	Virtually complete deficiency of VWF	<5	<5	Not applicable	↓↓ <10 IU/dL	Absent	Absent

↓ refers to a decrease in the test result compared to the laboratory reference range.

* <30 IU/dL is designated as the level for a definitive diagnosis of VWD; patients with symptomatic bleeding who have levels of VWF:RCo or VWF:Ag of 30 to 50 IU/dL and are classified as “low VWF.”

†The VWF:Ag in the majority of individuals with type 2A, 2B, or 2M VWD is <50 IU/dL.

likelihood of finding a putative mutation in the *VWF* gene is low. Of note, more than 50% of individuals with VWF levels in the mildly decreased range (30 to 50 IU/dL) are asymptomatic. Therefore, simply the presence of plasma VWF levels between 30 and 50 IU/dL does not automatically define “low VWF.” Additionally, management of this subcohort remains a matter of debate. In general, the need and type of treatment depends on the personal history of bleeding and degree of hemostatic challenge.

Approximately 75% of cases of VWD type 1 result from mutations that exert a dominant negative effect by impairing the intracellular transport of VWF subunits and causing subsequent decrease in VWF secretion. A second recently identified mechanism is the rapid clearance of VWF from the circulation because of specific mutations in the *VWF* gene. Therefore, impaired secretion and increased clearance are likely the 2 most common molecular mechanisms that lead to VWD type 1. The variant of VWD type 1 that is due to increased clearance is called type 1C. Because VWF is synthesized on a 1:1 ratio with VWFpp, an alteration of the ratio in favor of the propeptide suggests increased VWF clearance. This, plus the presence of unusually large multimers, is indicative of VWD type 1C. Patients with VWD type 1C have a robust initial response to desmopressin, but they exhibit an abrupt VWF level decrease within 2 to 4 hours, placing them at high risk for delayed postoperative hemorrhage.

A consistent diagnostic criterion is difficult to achieve, as not all individuals who inherit a mutation in *VWF* show signs of clinical disease (a phenomenon known as low

penetrance), and not all individuals that inherit the same mutation show the same clinical signs (known as variable expressivity). Individuals with blood group O have 25% to 30% lower VWF levels compared with those who have blood group A, although this variability should not affect the way that the disease is diagnosed. Additionally, plasma VWF levels increase by 10% per decade of life and may normalize for a subset of patients with prolonged follow-up. However, it is unclear whether normalization of historically low VWF levels with age normalizes the bleeding phenotype, and therefore the management of such patients remains a matter of debate. Lastly, VWF is an acute phase reactant and plasma levels may be higher during conditions of stress, inflammation, exercise, pregnancy, and in women using oral contraceptives.

VWD type 2

VWD type 2 is characterized by qualitative defects in VWF due to mutations in the *VWF* gene that affect the interactions of VWF with many of its ligands. VWD type 2 is subclassified into type 2A (loss of intermediate- and high-molecular-weight multimers because of decreased multimerization or increased susceptibility to ADAMTS 13), type 2B (gain-of-function mutation resulting in spontaneous VWF platelet binding under physiologic shear conditions, leading to clearance of the highest-molecular-weight multimers and mild thrombocytopenia), type 2M (loss of function mutations that decrease the interaction of VWF with its platelet receptor and decreases ristocetin cofactor activity), and type 2N (mutations in VWF

causing reduced binding to FVIII, allowing for increased clearance).

VWD type 3

VWD type 3 is inherited in an autosomal recessive mode and is characterized by complete lack of VWF protein with undetectable VWF antigen assay (VWF:Ag) and ristocetin cofactor assay (VWF:RCo) levels, and resultant low FVIII:C levels (<10%), representing the steady state of FVIII in the absence of its VWF chaperone. Multimers are absent and the bleeding pattern is usually severe.

The clinical presentation of VWD includes mucocutaneous bleeding—specifically, easy and excessive bruising and bleeding from mucosal surfaces, including the nose, mouth, and gastrointestinal tract. The extent, location, and nature of bruising are important clinical points. Multiple bruises of various ages in a variety of locations are suggestive of a disorder of primary hemostasis. Epistaxis or oral-pharyngeal bleeding sufficient to result in anemia suggests the presence of a hemostatic disorder. Heavy menstrual bleeding, particularly at onset of menarche, also is suggestive of a mucocutaneous bleeding disorder. Excessive bleeding following procedures involving the mucus membranes may unmask a previously unknown bleeding disorder. The most common of these events include childbirth, oral surgery (including dental work), tonsillectomy or adenoidectomy, and sinus surgery. Some patients may present to the hematologist as a result of a documented family history of bleeding without an individual specific bleeding event. Less commonly, patients may present because of abnormal screening tests ordered before a planned procedure. Clinical manifestations may range from mild to severe. Type 3 VWD may be associated with similar bleeding events observed in severe hemophilia, likely because of the extremely low FVIII levels. Severe heavy menstrual bleeding resulting in early hysterectomy has been observed in women with all subtypes.

Diagnosis

Screening laboratory tests (CBC, PT, aPTT) have limited value when a diagnosis of VWD is suspected. Therefore, in clinical practice, in the face of a significant history of mucocutaneous bleeding, specific laboratory assays for VWD are required.

Diagnostic assays for VWD include quantitative measurement of VWF (VWF:Ag), the platelet-binding function (VWF:RCo, in which the agglutination of fixed platelets in response to patient plasma is measured in the presence of ristocetin), and the FVIII coagulation (FVIII:C). Also, the distribution of VWF multimers is used to differentiate subtypes.

Limitations exist with several of these assays. Both VWF and FVIII are acute-phase reactants and may increase 2 to 5 times above baseline because of a variety of conditions or circumstances, including (among others) infection, stress, and pregnancy. These increased levels elevate low baseline levels to within the normal range, obscuring diagnosis. Therefore, normal levels do not completely rule out VWD, especially in the face of a suspicious clinical history, and must be interpreted with caution. Performance of these assays requires an experienced coagulation laboratory, ideally with on-site processing and analysis as opposed to an experienced coagulation laboratory analyzing a “send-out” sample often drawn thousands of miles away. Because of the difficulty in ruling out this disorder with 1 normal evaluation, it is not uncommon for patients to undergo repeated testing. When local laboratory results are inconsistent, a useful strategy is to perform testing in a reference hemostasis laboratory. Finally, many preanalytic variables must be considered to accurately interpret laboratory testing. For example, refrigeration of whole blood samples before separation can result in reduced plasma VWF levels; in addition, platelet contamination of the separated plasma may result in protease-induced VWF alterations, causing decreased activity. Given these variables, the laboratory diagnosis should not be made on just 1 set of subnormal levels, but at least 2 unless there are clearly identified acute-phase reactant effects present “normalizing” at least 1 set of VWF levels.

VWF:RCo is widely used and has been accepted for decades as the gold standard for VWF activity. Important limitations of this assay include: (i) high coefficient of variation; (ii) a lower level of detection of 10 to 20 IU/dL, which makes the accurate diagnosis of type 2 VWD difficult in patients with low VWF:Ag, as the VWF:RCo/VWF:Ag ratio becomes difficult to determine; and (iii) potential for false-positive results with variants which impact the ability of the VWF to bind to ristocetin but do not affect VWF activity (eg, the D1472H variant in exon 28 of the *VWF* gene that results in a spuriously low VWF:RCo but does not affect VWF function). A newly developed functional assay (VWF:GP1bM) that studies the direct binding of VWF to platelets without the need for ristocetin may overcome these limitations. Currently, availability of this assay is limited in the United States.

Low-dose ristocetin-induced platelet aggregation (LD-RIPA) is used to identify abnormally increased binding of VWF to platelets, as occurs in type 2B and platelet-type VWD. VWF multimers usually are run on an agarose gel to evaluate the full range of molecular weight multimers present within the mature VWF molecule. Multimeric analysis is required to differentiate between various subtypes of

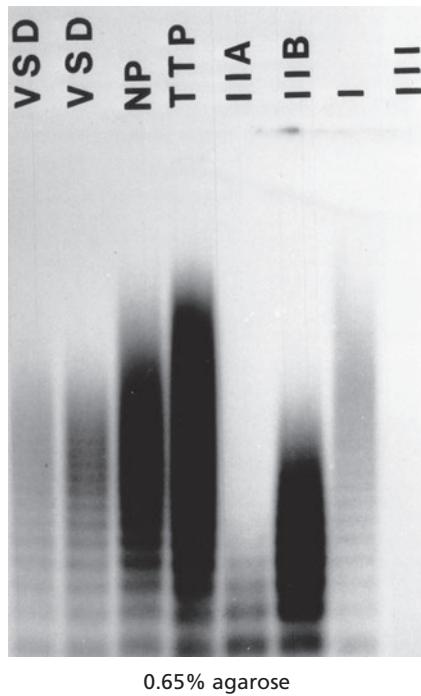


Figure 10-6 Representation of a VWF multimer analysis. The third column from the left represents normal plasma as indicated by the NP at the top of the column. In type 2A von Willebrand disease (VWD), there is a loss of high- and intermediate-weight multimers as indicated by the loss of the bands in the gel under the heading. In type 2B VWD, there is a loss of high-molecular-weight multimers (HMWM). In type 1, all the multimers are present but in reduced amounts, as can be seen by the presence of all the bands but with more faint staining than seen in normal plasma. In type 3 disease, there is a complete absence of multimers, and no staining of bands is visible. The labeled columns VSD and TTP stand for ventricular-septal defect, a condition that results in acquired von Willebrand syndrome (AVWS) with the loss of multimers of all sizes; and thrombotic thrombocytopenic purpura, in which ultralarge multimers can be observed.

VWD type 2, and their absence easily identifies VWD type 3 (Figure 10-6). The FVIII activity level and FVIII-binding assay provide a more accurate diagnosis of VWD type 2N. Finally, the collagen-binding assay measures binding of large VWF multimers to collagen and represents an additional method to assess VWF functional activity. This assay recently gained attention when it was reported that several families with abnormal bleeding symptoms have mutations in the VWF collagen-binding site with preserved VWF:Ag and VWF:RCo. The collagen-binding assay does not require the use of ristocetin, but studies have reported that the type of collagen employed influences the results.

Laboratory test results are compatible with VWD type 1 if the levels of both VWF:RCo and VWF:Ag are

<30 IU/dL and the plasma VWF multimer distribution is normal, though the intensity may be reduced due to lower amount of protein. Additionally, the VWF:RCo/VWF:Ag ratio approximates 1. In patients with VWD type 1C, the VWF:Ag and VWF:RCo levels are low, and in most cases the multimer assay is characterized by the presence of abnormally large high-molecular-weight forms. As this subtype is characterized by rapid VWF clearance, a VWFpp level allows for discrimination of VWD type 1C through the VWFpp/VWF:Ag ratio (in patients with VWD type 1C, the ratio is typically >3). As previously noted, patients with VWF:RCo and VWF:Ag levels between 30 and 50 IU/dL and bleeding symptoms are classified as “low VWF.”

VWD type 2 is a qualitative defect caused by mutations in VWF that result in abnormal interactions with several of its ligands. The diagnosis of type 2A is made in the presence of a low VWF:Ag and a disproportionately low VWF:RCo, with pronounced loss of HMWM. The VWF:RCo/VWF:Ag ratio is less than 0.6. Type 2M is caused by mutations in the platelet glycoprotein 1ba (GPIb) binding site, with resultant decreased binding of VWF to GPIb and subsequent impairment of platelet-dependent function. The multimer structure and distribution in VWF is normal. Type 2B results from gain-of-function mutations in the binding site for GPIb, leading to the formation of rapidly cleared platelet-VWF complexes. LD-RIPA is employed to confirm this subtype. A level of ristocetin insufficient to promote platelet binding with normal VWF causes enhanced platelet agglutination in these gain-of-function mutations. This phenomenon is also seen in patients with platelet-type VWD (also known as pseudo-VWD), a rare disorder caused by mutations in platelet GPIb. It is important to differentiate these 2 entities, as treatment approaches are significantly different. VWD type 2B is treated with VWF concentrates because the molecular defect is in VWF; whereas pseudo-VWD is treated with platelet transfusions because it is caused by mutations in platelet GPIb. For the evaluation of pseudo-VWD, the patient's platelets are tested with a normal exogenous VWF substrate in a ristocetin-induced platelet-agglutination-based mixing study. Enhanced binding confirms the diagnosis. Finally, type 2N is characterized by mutations in the FVIII-binding site of VWF, disturbing the normal interaction of these 2 proteins. Patients with VWD type 2N may exhibit normal or decreased VWF:Ag and VWF:RCo with disproportionately decreased FVIII:C, which may be misclassified as mild hemophilia A. Specific FVIII-binding assays are used to confirm the diagnosis of type 2N. Symptomatic patients are either homozygous or compound heterozygous for mutations in the *VWF* gene.

Patients with a prior diagnosis of mild FVIII deficiency who do not respond well to recombinant FVIII infusions or belong to families for whom the inheritance appears to be autosomal dominant should be evaluated for VWD type 2N.

VWD type 3 is characterized by undetectable VWF:Ag and VWF:RCo levels, FVIII:C levels commonly <10%, and lack of multimers. A description of the laboratory pattern for each subtype is shown in Table 10-1.

Genetic testing

Sequencing of the VWF gene is challenging due to its large size, highly polymorphic structure, and presence of a homologous partial pseudogene on chromosome 22. Additionally, identifying a genetic basis for VWD type 1, the most common variant, is particularly difficult, with 5 population-based epidemiological studies identifying a putative mutation in only ~ 65% of tested subjects. Therefore, gene sequencing for diagnosis is currently reserved for specific cases in which these test results likely contribute significantly to diagnosis and management, particularly in cases in which treatment options vary based on diagnosis. Genetic testing can be used to differentiate type 2B from pseudo-VWD, mild hemophilia from VWD type 2N, and occasionally to subclassify VWD type 2. Genetic testing may be also justified in VWD type 3 because large deletions may predispose to the development of inhibitory antibodies and anaphylactic reactions.

Acquired von Willebrand syndrome

Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder with clinical symptoms and laboratory abnormalities similar to congenital VWD. It is characterized by an older age at onset of bleeding symptoms, and a lack of family history of bleeding. While the exact pathophysiology is unclear, 5 distinct mechanisms have been proposed: (i) decreased production of VWF (eg, hypothyroidism); (ii) autoantibodies against VWF and immune complex formation (eg, systemic lupus erythematosus, Hashimoto thyroiditis); (iii) adsorption of VWF to tumor cells (eg, Wilms tumor, lymphoproliferative disorders); (iv) drug-mediated proteolysis of HMWM (eg, ciprofloxacin); and (v) increased proteolysis of HMWM under pathological high-shear-stress conditions (eg, congenital heart disease, aortic stenosis [Heyde syndrome], extracorporeal devices, mechanical valves). Treatment of underlying medical disorders, such as surgery and chemotherapy for Wilms tumor, replacement therapy for hypothyroidism, immune suppression for SLE, and surgical correction of cardiac defects, usually results in rapid resolution of symptoms.

Treatment

The principle for management of VWD is to increase or replace VWF to achieve hemostasis. This is accomplished with either medications that cause the release of endogenous stores of VWF into the circulation (desmopressin) or the use of recombinant or plasma-derived VWF concentrates.

Mild to moderate bleeding associated with VWD type 1 often is managed with desmopressin, most commonly with the intranasal preparation, and antifibrinolytic agents as required for mucosal-based surgery or bleeding. Desmopressin's mechanism of action is based on the secretion of stored VWF from Weibel-Palade bodies in endothelial cells into the plasma. A desmopressin challenge test, as described in the "Platelet Function Disorders" section in this chapter, should be performed to document a hemostatic response in VWD. The VWF:Ag, VWF:RCo, and FVIII levels are performed before and 60 to 90 minutes after the dose (depending on the route of administration). Repeat laboratory evaluation at 4 hours postdose may be appropriate when an altered half-life of the native protein is suspected, as observed in type 1C. Approximately 80% of patients with VWD type 1 respond with hemostatic levels; however, the response varies and should be measured to determine its adequacy for specific hemostatic challenges. Repeated administration of desmopressin in proximity may lead to tachyphylaxis, with decreased response levels with repeated use likely resulting from depletion of the storage pool. Repeated doses also increase the risk of hyponatremia. Thus, use of desmopressin no more than once daily and for no more than 2 or 3 consecutive days serves as an acceptable clinical guideline for home use. To avoid hyponatremia, patients should be instructed to limit their fluid intake for 24 hours after desmopressin use. There are some reports of the benefits of desmopressin in VWD type 2; in general, it is less effective in these subtypes and has been reported to precipitate thrombosis or result in significant thrombocytopenia as a result of in vivo platelet aggregation in type 2B or platelet-type VWD. However, it can be useful in some patients with types 2M and 2A and in some genotypes of Type 2B. Desmopressin is ineffective in type 3 VWD and treatment is dependent on the use of replacement therapy via concentrate.

Several products available in the United States contain intact VWF, including Humate-P (CSL Behring, King of Prussia, PA), Alphanate (Grifols Biologicals, Los Angeles, CA), Koate DVI (Talecris, Research Triangle Park, NC), and Wilate (Octapharma, Lachen, Switzerland), with similar products available in other countries. These plasma-derived concentrates contain VWF and FVIII in varying ratios and with variable amounts of multimer size or distribution.

Humate-P, Alphanate, and Wilate are approved by the FDA for the treatment of VWD. Although these products are manufactured via processes that include viral attenuation and inactivation steps, a theoretic risk of transmission of infectious agents exists. Recently, a recombinant VWF, Vonvendi (Shire, Bannockburn, IL) was approved by the FDA for management of VWD in adults. Given that this product contains no FVIII, it is recommended that patients with FVIII levels <40% receive concomitant recombinant FVIII with the first dose of Vonvendi. Subsequently, the drug may be administered exclusively as endogenous FVIII production maintains hemostatic levels of FVIII within 6 hours of the first infusion of Vonvendi.

Antifibrinolytic agents are useful adjunctive therapies to both desmopressin and VWF concentrates and are used in a similar fashion as described for platelet defects. Contraceptive agents, including oral and levonorgestrel-IUD, can be effective therapies for the management of heavy menstrual bleeding. Topical measures also are useful in some situations. The benefits and risks of these agents are identical to those described in the “Treatment” section of the “Platelet function disorders” section in this chapter. Case reports exist in the literature regarding the use of rFVIIa in VWD; these are limited to patients with type 3 disease with inhibitors to VWF and patients with AVWS.

In addition to treatment with hemostatic agents, further aspects of care include anticipatory guidance and lifestyle modifications, education of patients and primary care providers, and use of local measures for management of mild bleeding. These approaches are similar to those in the preceding section on management of platelet disorders.

Gaps in knowledge

The most challenging aspect in the management of VWD is the establishment of an accurate diagnosis, particularly in type 1 disease. This can be especially difficult because VWF levels may appear to be normal because of the associated clinical circumstances, despite a clinical history suggestive of this disorder. Recently published data used a Bayesian analysis of laboratory data and personal and family history to predict the probability of diagnosis of VWD. Future research aimed at the development of laboratory assays with improved performance characteristics to decrease variability and diagnostic dilemmas is needed. A wide variation in bleeding symptoms exists among patients within the same disease subtype, likely because of genetic modifiers of the bleeding phenotype. Overall, currently available therapies are effective; however, it is not completely clear under what circumstances specific therapies are best applied to achieve an optimal outcome. There are

few prospective comparative therapy studies to guide physicians in determining the risks and benefits of available therapies. Published treatment guidelines published by the National Heart, Lung, and Blood Institute, as well as more recent ones published by the United Kingdom Haemophilia Centre Doctors’ Organisation, are based on the best available evidence and expert opinion.

KEY POINTS

- VWD is the most common inherited bleeding disorder in the general population.
- VWD is divided into several subtypes. Type 1 is the most common, encompassing two-thirds of cases.
- Laboratory diagnosis of VWD may be difficult, especially in type 1.
- VWD treatment is based on the subtype; the most common agents used for treatment include desmopressin, antifibrinolytics, hormonal therapy for heavy menstrual bleeding, and VWF concentrates for severe bleeding or in types 2 and 3.

Disorders of secondary hemostasis

Congenital hemophilia A and B (FVIII and FIX deficiency)

Pathophysiology

The previous review of the physiology of hemostasis reveals the critical roles played by FVIII and FIX in thrombin generation and ultimately normal fibrin clot formation. Absence or decreased amounts of either FVIII or FIX results in reduced thrombin generation on the surface of activated platelets at injured sites. Inadequate thrombin generation leads to fibrin clots with poor structural integrity, as visualized by electron microscopy; specifically, formation of large, coarse fibrin strands as opposed to normal thinner strands that form a tight network is observed. In addition, reduced thrombin generation results in decreased activated FXIII, which is required for cross-linking of fibrin monomers and decreased TAFI generation, both of which result in a clot less resistant to normal lysis. Therefore, deficiencies of FVIII or FIX result in poorly formed clots that are more susceptible to fibrinolysis, clinically observed as the bleeding manifestations in hemophilia.

Etiology

Congenital deficiencies of FVIII and FIX occur as a result of genetic mutations in *F8* and *F9*, respectively, both located on the long arm of the X chromosome. Accordingly, these deficiencies are most commonly observed in

males due to their hemizygous state. In women and girls, a range of factor levels can be observed; though rarely, levels in the severely or moderately deficient range can occur because of skewed X-chromosome inactivation or the presence of other genetic abnormalities, such as Turner syndrome or X-autosomal translocations. A wide range of mutations result in hemophilia, and the mutation type (deletion, inversion, missense, or nonsense) and specific area of the protein affected determines the severity of disease. In approximately 25% of cases, no family history is identified. In such cases, the affected individual's mother is either not a carrier and the de novo mutation arose after conception of the affected male child or is a carrier as a result of a germline mutation at the time of the mother's conception.

Although over 2,100 unique mutations have been associated with hemophilia A, the most common mutation, occurring in up to 45% of patients with severe hemophilia A, is the intron 22 inversion. The inversion is caused by homologous recombination between the 9.5 kb sequence within intron 22 of the *F8* gene and 1 of 2 extragenic homologous regions. As a result, exons 1 to 22 are inverted and separated from exons 23 to 26. A wide variety of causative *F8* gene mutations have been reported, including small and large deletions, and missense, nonsense, and splice-site mutations in nearly all of the coding areas of *F8*. Over 1,100 unique mutations have been associated with hemophilia B. In contrast to hemophilia A, while there is not one predominant gene defect in hemophilia B, missense mutations predominate.

Clinical presentation

The clinical presentation of congenital hemophilia is highly variable and correlated with the level of deficiency. In infants born to known carriers, the diagnosis most often can be established at birth by assaying FVIII or FIX from umbilical cord blood. Of note, FIX levels are physiologically low in the neonatal period. Accordingly, a low FIX level in the cord blood needs to be repeated at 6 to 12 months before a diagnosis of hemophilia B can be confirmed. Prenatal testing is available if the genetic defect has been identified within the family. The presentation of symptoms leading to diagnosis in patients either without a family history or not tested at birth is quite variable and dependent on the severity of disease.

Severe hemophilia, defined as a factor activity level <1%, may present in the newborn period with intra- or extracranial bleeding; prolonged bleeding from venipuncture, heel stick or after circumcision; or with excessive bruising. Infants with severe hemophilia who do not develop symptoms in the newborn period often present during

the first year of life with abnormal bruising, muscle hematoma (especially with immunization), or bleeding in the joint or muscle due to activity or intercurrent injury. Although the precise prevalence of intracranial hemorrhage is not known, it likely approximates 1% to 3%. Assisted delivery is associated with bleeding in the neonatal period and maternal awareness of carrier status may result in lower use of assisted delivery devices (forceps/vacuum) and, in turn, lower rate of intracranial hemorrhage. Moderate hemophilia (factor activity levels between 1% and 5%) has a variable age of presentation; diagnosis may be established due to a known family history, in the newborn period due to bleeding, or later in life (even as an adult) with a bleeding event associated with intercurrent injury or invasive procedure. Bleeding symptoms include deep tissue, muscle, or joint bleeding; mucocutaneous bleeding is a common presentation due to fibrinolysis in the oropharynx and the inability to form a stable clot. Mild hemophilia (factor activity levels between 5% and 40%) may be diagnosed at ages similar to moderate hemophilia. In the absence of a family history, patients with mild hemophilia typically present later in childhood or during the teenage or adult years with bleeding associated with injury or surgery rather than spontaneous hemorrhage.

Diagnosis

In the absence of a known family history of hemophilia, in which case direct assessment of FVIII or FIX in accordance with the family history is most appropriate, the laboratory diagnosis of hemophilia begins with screening coagulation studies, including PT and aPTT; the aPTT is almost always abnormal. However, it is important to be cognizant of circumstances in which the aPTT may be normal, especially in mild deficiencies (Figure 10-7). After identification of a prolonged aPTT, a mixing study with normal plasma is performed. Correction of the prolongation into the normal range points to a factor deficiency. Specific factor assays are used to identify the deficient factor. In the setting of an isolated prolonged aPTT, FVIII, FIX, and FXI should be assayed. There are 2 methods by which the factor activity can be measured: a 1-stage assay and a 2-stage chromogenic assay. The 1-stage assay is based on the principles of the aPTT, in which plasma is combined with phospholipid, calcium, and a contact activator (eg, kaolin, silica, ellagic acid) and the time it takes to form a fibrin clot is measured. When measuring specific factor activity in a 1-stage assay, the test plasma is serially diluted in plasma that is deficient in the clotting factor of interest (so that all other clotting factors are in excess), and an aPTT is performed. The results should form a line that is parallel to a line made for the standard reference sample

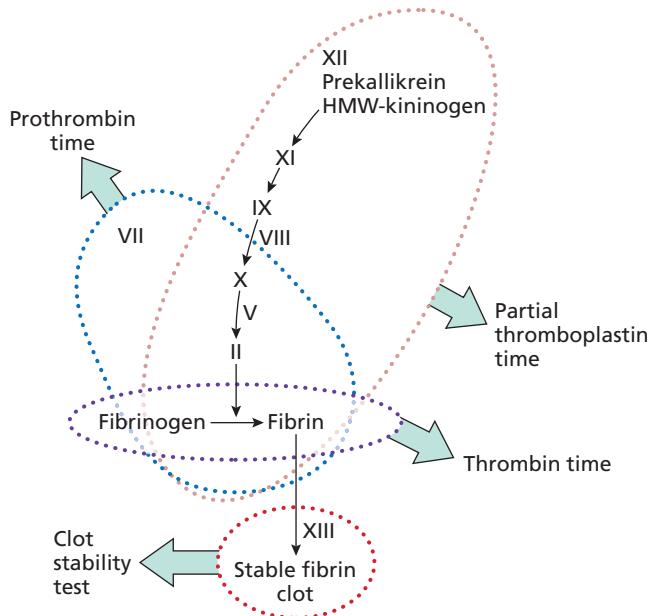


Figure 10-7 Plasma coagulation reactions in in vitro laboratory assays. Factor XII, prekallikrein, and high-molecular-weight kininogen are required for a normal activated partial thromboplastin time but not for normal in vivo hemostasis. This diagram outlines the coagulation factors required for each of 4 basic tests that characterize the coagulation cascade: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time, and FXIII assay.

from which the level of the factor of interest is determined. The 2-stage method involves an incubation step to generate FXa and a second step to measure the FXa, typically by its cleavage of a chromogenic substrate rather than formation of a clot. The amount of FXa generated is proportional to the amount of FVIIIa/FIXa available. Discrepancies between these 2 measures have been identified in up to one-third of patients with mild and moderate hemophilia A. The reason for the discrepancy lies in the underlying *F8* mutation. Mutations that affect FVIII cleavage by thrombin result in higher FVIII levels in the 2-stage assay because the incubation period allows for more FVIII cleavage by thrombin to occur. Conversely, mutations that reduce FVIII stability have lower 2-stage FVIII levels compared with 1-stage levels, due to degradation of the FVIII during the incubation period. As a result, some have urged that both assays be performed for the diagnosis of hemophilia A. At a minimum, one needs to consider performing the alternative FVIII assay if the clinical picture is not consistent with the assay result.

Ultimately, the type and severity of hemophilia are established based on the FVIII or FIX assay. As previously discussed, appropriate specimen procurement and handling are critical to obtaining accurate results. In new-

borns, where cord blood is tested due to a known family history, levels may be altered based on sample procurement, level of deficiency, and neonatal variations, as seen with decreases in vitamin K-dependent clotting factors. Therefore, repeat testing may be required based on cord blood results and their concordance with expected results and clinical symptoms. In addition, assaying factor activity levels at the lowest range of the curve is technically difficult, and sample analysis through a reference laboratory may aid differentiation of the severe from moderate forms. Finally, because FVIII is an acute-phase reactant, obtaining a true baseline level may be difficult in patients with moderate and mild deficiencies when tested in the setting of inflammation.

Treatment

The mainstay of hemophilia treatment is replacement of the deficient coagulation factor. There are a number of commercially available factor concentrates to treat both FVIII and FIX deficiency (Tables 10-2 and 10-3). The choice of the specific product used includes consideration of (among other things) availability, cost, method of manufacture, delivery system, and half-life. Theoretical concerns include infectious agent transmission and inhibitor development.

Both recombinant and plasma-derived products are available, and decisions about which product to use should be made in consultation with the patient and family. Recently, FVIII and FIX products have undergone modifications to extend their half-life—such as fusion to FC fragment, albumin, or polyethylene glycol (PEG). Fusion with an FC fragment or albumin takes advantage of the FC receptor and leads to recirculation of the fused FVIII or FIX. These alterations have led to an approximately 1.5-fold increase in FVIII half-life, though interindividual variation exists, and a 4- to 5-fold increase in the FIX half-life. Pegylation increases the size of the molecule in circulation, which has also been a more effective strategy for FIX than FVIII. Efforts to extend the half-life of clotting factors have been less successful with FVIII than FIX because FVIII is already a large molecule that is predominantly intravascular and its clearance is dictated by VWF. This group of products is referred to as extended half-life (EHL) products. In the absence of a clear consensus definition, some products without specific modification to extend the half-life have claimed to be an EHL because this label is advantageous when marketing to patients and prescribers alike. When evaluating whether a product has a longer half-life, it is important to compare the half-life of the new product to the studied comparator. Patient characteristics (eg, age and coexisting liver disease) can influence

Table 10-2 Factor VIII concentrates currently available in the United States

Brand name	FVIII	Modification	t _{1/2} (h)	Comparator (t _{1/2} [h])	Production cell line	Stability at RT (mo)
Plasma-derived						
Monoclate	Full-length	-	~12	-	NA	None
Hemophil M	Full-length	-	~12	-	NA	None
Recombinant standard half-life						
Recombinate	Full-length	-		-		None
Kogenate FS	Full-length	-	13.7	-	BHK	3
Advate	Full-length	-	12	-	CHO	6
Xyntha	B-domain deleted	-	11.2	-	CHO	6
Novo-8	B-domain truncated	-	10.8	-	CHO	6
Kovaltry	Full-length	-	14.3	Kogenate (12.2)	BHK	12
Nuwiq	Full-length	-	17.1	Kogenate (bioequivalent)	HEK	3
Afstyla	Single chain	-	14.5	Advate (13.3)	CHO	3
Recombinant extended half-life						
Eloctate	B-domain deleted	FC-fused	19	Advate (12.4)	HEK	6
Adynovate	Full-length	Random pegylation	14.3	Advate (10.4)	CHO	1

Other FVIII concentrates approved for use in FVIII deficiency; these may also contain VWF and are not in general use.

FVIII, factor VIII; BHK, baby hamster kidney; CHO, Chinese hamster ovary; HEK, human embryonic kidney; NA, not applicable; RT, room temperature.

the half-life, thus comparisons between products need to be made in the same patient population.

For all products, 1 IU/kg of FVIII typically increases the FVIII level by 2%. Infusions of nonmodified FVIII products can be repeated as needed approximately every 8 to 12 hours. Dosing of EHL products during acute bleeding events varies and should be performed according to prescribing information. With FIX, dosing depends on the product used. Plasma-derived FIX (pdFIX) and FIX-FC both increase the FIX level by 1% for each IU/kg infused, whereas with rFIX, the level increases by 0.6% to 0.8%, with children exhibiting a lower recovery compared with adults. Nonmodified FIX (rFIX and pdFIX) products can be repeated every 12 to 24 hours as needed for acute bleeds. As with FVIII EHLs, FIX EHLs for treatment of acute bleeding should refer to the specific product's prescribing information. With any product, the treatment goal for severe bleeding events is to keep the FVIII/FIX level in the normal range until the bleed has resolved.

Treatment approaches are divided into 2 main categories: prophylaxis and on-demand. Prophylaxis is the regular infusion of factor replacement therapy to prevent bleeding events. Primary prophylaxis is defined as the initiation of regular, continuous factor replacement therapy started before or shortly after the first hemarthrosis and before the age of 3 years, has been proven to be the most

Table 10-3 FIX concentrates currently available in the United States

Brand	Modification	Recovery (IU/kg per IU/dl)	Half-life (h)	Cell line
Plasma-derived				
Mononine	-	1.0	22.6	NA
Alphanine	-	NA	21	NA
Recombinant standard-half-life				
Benefix	-	0.7	19.4	CHO
Ixinity	-	0.98	24	CHO
Rixubis	-	0.9	25.4	CHO
Recombinant extended-half life				
Alprolix	FC-fused	1.12	97	HEK
Rebinyn*	Glycopegylated	2.34	83	CHO
Idelvion	Albumin-fused	1.3	104	CHO

NA, not available/applicable; CHO, Chinese hamster ovary; HEK, human embryonic kidney; *, indicated for on-demand therapy only.

effective approach to prevent the development of joint disease. Therefore, primary prophylaxis is considered the standard of care for patients with severe hemophilia. Full-dose prophylaxis entails the administration of standard half-life factor concentrates every other day for hemophilia A, or twice a week for hemophilia B. This regimen

is time- and resource-intensive and often requires central venous access in younger children. An alternative approach to full-dose, primary prophylaxis is to use escalating dose and frequency of prophylaxis. Such an approach starts patients on once-a-week factor infusions and escalates therapy based on bleeding symptoms. Once primary prophylaxis has been instituted, most need to continue indefinitely. A subset of patients (~25%) may be able to discontinue prophylaxis in adulthood while maintaining a low rate of bleeding.

Secondary prophylaxis is the regular infusion of factor replacement initiated after the second hemarthrosis, but before the presence of joint disease on physical examination or imaging studies. The goal is to interrupt a bleeding pattern and prevent joint damage through suppression of bleeding episodes. Tertiary prophylaxis is when regular infusions of factor replacement are started after the onset of joint disease seen on examination or imaging studies. Studies of tertiary prophylaxis have demonstrated improvements in bleeding frequency, quality of life, and joint examination. Joints with repeated bleeding develop acute or chronic synovitis, followed by articular damage; the process of repeated bleeding (3 or more bleeds during a 6-month period) in a joint is termed *target joint*. The bleeding pattern in target joints has been documented to be amenable to prophylaxis. Administration of factor concentrates to prevent bleeding only prior to circumstances that place patients at high risk for bleeding, such as before sports, may be useful in those unwilling to do continuous prophylaxis or for those with nonsevere disease who historically bleed when participating in these types of activities. In other situations, limited prophylactic therapy is reasonable and is reviewed in cited references.

Although primary prophylaxis is used most frequently in patients with severe disease, some individuals with moderate hemophilia require this therapy because of their bleeding pattern. The best dose for prophylaxis varies according to a variety of factors, which include but are not limited to the product used, the age of the patient (younger patients have low recovery and shorter half-life), and the patient's joint status and activity level. Although prophylaxis is effective in the prevention of the majority of spontaneous bleeding events, patients who experience breakthrough bleeding episodes require immediate treatment.

Episodic treatment for bleeding episodes is referred to as on-demand therapy (ie, the use of factor replacement therapy after bleeding occurs). This treatment approach does not require regular infusions with their associated issues (cost and need for central venous access) and is less expensive in the short run but ineffective at preventing

joint disease. This mode of therapy now is used primarily for patients with moderate and mild hemophilia due to the infrequency of bleeding events and the associated low risk of joint disease. Rarely, patients with severe hemophilia have infrequent bleeding events and can be managed with on-demand therapy, with or without prophylactic infusions prior to specific activities. The typical initial dosing for bleeding episodes targets peak levels of 30% to 50% for treatment of a mild bleeding episode and levels of 80% to 100% for a severe bleeding episode. Treatment is continued until the bleeding event resolves, which could be 1 infusion with a mild bleeding event or many days for more significant bleeding events, such as a large muscle hematoma. Infusion therapy for hemophilia, regardless of the regimen used, is best delivered in the home setting to allow for prompt therapy (within 2 hours of bleeding onset). Family members and patients should be trained to administer the factor replacement therapy at home via peripheral venipuncture or central venous line without the need for a medical facility.

Adjunctive therapy for hemophilia is similar to that discussed for platelet defects and VWD. Patients with mild hemophilia A may be treated with desmopressin after a challenge dose demonstrates a hemostatic response; the response level dictates the type of bleeding events that may be treated with this agent. Antifibrinolytic agents are efficacious for mucosal bleeding and commonly are used in conjunction with factor replacement or desmopressin. For women with hemophilia who experience heavy menstrual bleeding, hormonal therapy including the levonorgestrel IUD can be used, as well as antifibrinolytic therapy.

Complications of treatment: inhibitors

A significant complication of hemophilia after exposure to replacement therapy is the development of neutralizing antibodies that bind FVIII/FIX, termed *inhibitors*. Inhibitors render standard treatment with replacement therapy ineffective and result in hemorrhagic episodes that are prolonged and more difficult to control, with associated increased risk of morbidity. The incidence of inhibitors is between 20% and 35% in severe, previously untreated, FVIII-deficient patients; 13% in nonsevere FVIII-deficient patients who receive FVIII replacement therapy; and <5% in severe FIX-deficient patients. The present inhibitor prevalence is approximately 10% in FVIII deficiency and 3% to 5% in FIX deficiency. Risk factors for inhibitor development are both characteristics of the patient and how factor replacement therapy is delivered. Because of the greater prevalence of hemophilia A and higher incidence of inhibitors among patients with hemophilia A, more is

known about risk factors for inhibitor development in this population. Among the patient-specific risk factors, the most important is hemophilia severity, with patients with severe disease at highest risk. The specific genetic mutation, ethnicity, and family history of inhibitors also have been shown to affect the expression of this complication. Mutations resulting in major disruptions of the *F8/F9* genes, such as large deletions, are associated with increased risk. Genetic polymorphisms of immune response genes (*IL10*, *TNF-alpha*, and *CTLA-4*) have also been associated with inhibitor risk. In addition, patients of African or Hispanic ethnicity have a significantly higher rate of inhibitor development. Treatment-related risk factors include the source (plasma-derived vs recombinant) of the factor product used. In a randomized clinical trial, 264 previously untreated patients with severe hemophilia A were randomized to either a plasma-derived or recombinant product. In the 251 analyzed patients, an inhibitor occurred in 26.8% (high titer, 18.6%) that received plasma-derived products vs 44.5% (high titer, 28.4%) of patients that received recombinant products. Other treatment-related risk factors include receipt of intensive replacement therapy (5 or more consecutive days) or surgery during early factor exposure. Inhibitor development in hemophilia B is far less common and has associated unusual complications. Patients with FIX deficiency may develop anaphylactoid reactions to infused FIX concentrate before or at the time of inhibitor emergence.

Inhibitors are divided into 2 categories: low titer (also known as low-responding inhibitors) and high titer (high-responding inhibitors). A low-responding inhibitor is characterized as one with a titer measured in the Bethesda assay of <5 Bethesda units (BU)/mL despite repeated exposure to factor replacement, whereas high-responding inhibitors are those that achieve a titer >5 BU/mL at any time, regardless of present titer. Patients with high-responding inhibitors may exhibit a decrease in, or an undetectable inhibitor titer with complete withdrawal of, the specific clotting factor. Despite this, with subsequent exposure to the deficient factor, these patients mount a memory response and demonstrate an increase in inhibitor titer in 7 to 10 days after exposure. The term for stimulation and increase of inhibitor titer is *anamnesis*. Therefore, it is clear that high-responding inhibitor patients who achieve an undetectable inhibitor titer have not had the inhibitor response ablated and should not be challenged again unless experiencing life- or limb-threatening bleeding episodes or if there is a plan for inhibitor eradication with immune tolerance induction (ITI).

Patients with low-responding inhibitors commonly are managed with higher doses of factor replacement therapy

to overcome the inhibitor titer and achieve a hemostatic factor level. Approximately 10% of low-titer inhibitors resolve without intervention (often within a few weeks) and are termed *transient inhibitors*; therefore, ongoing measurement of titers is important to document persistence.

The 3 important strategies for the management of patients with high-responding inhibitors include: (i) management of bleeding episodes, (ii) prevention of bleeding, and (iii) eradication of the inhibitor. The management of bleeding episodes in inhibitor patients is challenging, with the majority of hemophilia-related morbidity in the United States occurring in patients with high-responding inhibitors. Bypassing agents are used to treat bleeding episodes in patients with high-responding inhibitors because they are not able to achieve hemostatic clotting factor levels with factor concentrates. Two bypassing agents are available for the management of bleeding in inhibitor patients: activated prothrombin complex concentrate (APCC) (FEIBA; Baxter, Deerfield, IL) and rFVIIa (NovoSeven; Novo Nordisk, Bags-vaerd, Denmark). APCC is a plasma-derived concentrate consisting of the vitamin K-dependent clotting factors both in nonactivated and activated forms. The mechanism of action of APCC largely is ascribed to the presence and action of FXa and prothrombin, although FIXa and FVIIa also are contained; small quantities of nonactivated FVIII may be present. rFVIIa contains FVIIa as its sole agent and is genetically engineered. The main mechanism of action of rFVIIa in patients with hemophilia is through tissue-factor-independent thrombin generation on the surface of activated platelets. Both APCC and rFVIIa have been demonstrated to be safe and effective, with variable response rates ranging from 70% to 90%. Two prospective studies compared these products and revealed essentially similar response rates. Both products have considerable data supporting their safety (>30 years for APCC and >10 years for rFVIIa), with few reported thrombotic events in hemophilic inhibitor patients. In addition, APCC as a plasma-derived product has an excellent safety record without documented viral transmission.

The most important consideration when choosing a product in an inhibitor patient is its ability to achieve rapid bleed control and thereby limit morbidity and mortality. Thus, product choice is individualized. Because APCC is an FIX-based product, its use in FIX inhibitor patients with infusion-associated reactions is contraindicated. APCC may contain small quantities of FVIII and result in continued stimulation of the inhibitor titer in FVIII-deficient patients. Accordingly, rFVIIa that does not contain FVIII or FIX does not lead to anamnesis and may be preferred if trying to allow the inhibitor to reach

Table 10-4 Typical dosing for currently available bypassing agents

Agent	Joint/muscle	Life or limb threatening	Preoperative	Prophylactic
APCC*	50–75 U/kg	66–100 U/kg	50–100 U/kg [‡]	85 U/kg every other day
	Repeat every 8–12 hours as needed	Repeat every 8–12 hours		
rFVIIa	90–120 µg/kg	90–120 µg/kg	90–120 µg/kg	90 µg/kg/d
Standard dose [†]	Repeat every 2–3 hours as needed	Repeat every 2–3 hours	Repeat every 2 hours [‡]	
rFVIIa	270 µg/kg	270 µg/kg	No data	270 µg/kg/d
High dose	Data not available on follow-up doses required	Data not available on follow-up doses required		

*Doses of >200 U/kg/d are contraindicated per prescribing information.

[†]The licensed dose of rFVIIa in the United States is 90 to 120 µg/kg for treatment and prevention of bleeding during surgery; not approved for prophylaxis.

[‡]Frequency and duration vary according to the type of surgery. Refer to prescribing information.

APCC, activated prothrombin complex concentrate.

a low level before initiation of ITI. Management of acute bleeding is critical; therefore, inhibitor stimulation is not an absolute contraindication to APCC use during this time if any bleeding episode is unresponsive to rFVIIa. Dosing regimens for both products have been established (Table 10-4). Occasionally, patients present with bleeding events refractory to both agents. In such cases, the use of combination APCC and rFVIIa has been reported using an alternative sequential regimen. Alternatively, another approach is to adjust APCC or rFVIIa dosing based on results of global hemostatic assays (thrombin generation assay and thromboelastography). Although these approaches have been demonstrated to be effective and safe in a small number of young children, the reports remain anecdotal.

Historically, the prevention of bleeding in inhibitor patients has been more challenging. Several prospective studies have demonstrated the successful use of rFVIIa for both minor and major surgery (see Table 10-4 for dosing recommendations). This has led to an increased availability of required surgical procedures in inhibitor patients, most notably orthopedic procedures for amelioration of hemophilic arthropathy. APCCs have been used in the surgical setting, but the body of reports supporting their use, dosing, and safety is smaller compared with rFVIIa. It is important to mention that the risk of thrombosis may increase with the sequential use of rFVII and APCCs.

Routine prophylaxis with bypassing agents to prevent bleeding episodes in inhibitor patients has become more common. Several studies have demonstrated its utility. APCC (85 U/kg, 3 to 3.5 times per week) has demonstrated a 62% to 72.5% reduction in the frequency of bleeding events. However, the response was variable amongst patients,

with up to 38% having minimal to no change in bleeding frequency. rFVIIa prophylaxis was studied at 2 doses—90 and 270 µg/kg daily—and led to a 47% and 68% reduction in bleeding frequency, respectively. Although prophylaxis with bypassing agents has demonstrated benefit, it is less than that seen with tertiary prophylaxis using factor replacement therapy in noninhibitor patients and is much more difficult to achieve, given that APCC is typically a large infusion volume and rFVIIa is dosed frequently. The introduction of emicizumab (Hemlibra; Genentech, South San Francisco, CA) has introduced the opportunity for effective prevention of bleeding. Emicizumab is a bispecific antibody that binds both FIXa and FX, bringing FIXa in physiological proximity with FX to facilitate FXs activation. In a sense, emicizumab is able to mimic the cofactor action of FVIIIa. In recent clinical trials, >60% of adults and children treated with emicizumab had no bleed events during the 6-month study period. Of the 24 adults and 13 children that had pretreatment bleeding data available as part of a noninterventional study, only 2 adults failed to show an improvement in bleed rate. Treatment of breakthrough bleeding for patients on emicizumab should be undertaken cautiously. Multiday dosing of APCC was associated with thrombosis and thrombotic microangiopathy in 5 patients. Treatment with rFVIIa alone was not associated with thrombotic microangiopathy, though most treatments were confined to less than 24 hours. Emicizumab is currently approved for prevention of bleeding in patients with hemophilia A and inhibitors and trials in patients without inhibitors are ongoing.

Although emicizumab provides the opportunity to effectively and efficiently prevent bleeding, inhibitor eradi-

cation remains an important consideration in order to restore the capacity to use FVIII for treatment of bleeding. Inhibitor eradication with ITI requires regular administration of the deficient factor to reset the immune system by inducing peripheral tolerance. Hay et al completed and published an international prospective ITI study in good-risk patients. This study compared daily high-dose FVIII (200 IU/kg) to low-dose FVIII (50 IU/kg) 3 times weekly. The study was stopped before reaching the planned endpoint because of an increased rate of bleeding observed in patients receiving FVIII 50 IU/kg 3 times weekly. Typical ITI regimens may include either of these infusion schedules or a regimen of 100 IU/kg given once daily. Clinical studies have identified several factors associated with ITI success, including the historical peak inhibitor titer (<200 BU/mL), titer at start of therapy (<10 BU/mL), peak titer after the start of ITI (<100 BU/mL), age at initiation (<8 years), and time from inhibitor development to ITI start (<2 years). It is best to initiate ITI when the titer is <10 BU/mL, although this must be balanced against the risk of delaying tolerance and persistent risk of bleeding. For those who fail an initial course of ITI, the rate of success with a second ITI course is unknown. Options to consider include using a VWF-containing FVIII product or adding rituximab, though clear evidence to guide treatment decisions is lacking. The best approach to inhibitor eradication in patients with nonsevere hemophilia is also unclear. In general, patients with nonsevere hemophilia do not respond to ITI as well as patients with severe disease. Rituximab without ITI has also been used and may lead to more rapid inhibitor eradication than observation alone.

Because of the associated risk of allergic reactions in patients with hemophilia B, ITI may not be possible or, if undertaken, requires desensitization to FIX. FIX-deficient patients with inhibitors undergoing ITI are at risk for developing nephrotic syndrome. ITI-associated nephrosis is more likely to occur in patients with a history of an anaphylactoid reaction. The etiology of nephrosis in these patients is unclear, although it is thought to be related to immune complex formation. The overall success rate of ITI in FIX deficiency is 35%, far lower than the 75% achieved in FVIII deficiency. Thus, although fewer FIX inhibitor patients exist, they are a significant treatment challenge for practitioners.

Prognosis and outcomes

Currently, patients with severe hemophilia without inhibitors, HIV, or HCV treated on a prophylactic regimen have an excellent prognosis and lead near-normal lives.

The Swedish cohort followed for nearly 40 years substantiates these outcomes. For patients with inhibitors, the outcome is more variable, and the risk of morbidity is significant. When ITI is successful, the outcome can be converted to that of a noninhibitor patient, yet the morbidity experienced depends on the amount of joint disease and other bleeding events that occurred before ITI success. It is likely that many of these patients have experienced hemarthroses, muscle, or even intracranial hemorrhage and that some of these bleeding events are associated with permanent sequelae. For inhibitor patients in whom ITI was not successful or not performed, significant musculoskeletal morbidity is common, resulting in permanent disability and poor quality of life. With improved hemostatic coverage available for surgical interventions, even hemophilic patients with inhibitors now may undergo procedures to reduce pain and increase functionality. Combined with the increased use of prophylaxis, it is possible now to develop treatment strategies to ameliorate the consequences of recurrent bleeding and allow patients to lead more productive lives.

Gaps in knowledge

The greatest challenge with the potential for significant reward lies with gene therapy, a potentially curative approach. Early clinical trials using adenoviral vectors have demonstrated the ability of FIX or FVIII gene transfer to increase FVIII or FIX levels and reduce bleeding and factor consumption. One approach deserving of future work is the prevention of inhibitor formation. An improved understanding of the immunologic pathways involved in inhibitor formation and development of tolerance would open avenues to prevent inhibitor development or increase the rate of tolerance achieved. It is conceivable that an approach could be developed to program the immune system to induce tolerance before or in association with exposure to exogenous normal factor concentrate. Future research efforts could lead to the development of replacement products that are less, or perhaps not at all, immunogenic. In inhibitor patients, methods to perform ITI in FIX deficiency lag behind those for FVIII deficiency. For patients with anaphylactoid reactions, options for desensitization and subsequent ITI are limited, with an overall poor outcome, although rare successes have been reported. The FIX-deficient inhibitor population with anaphylactoid reactions represents a small vulnerable population with only 1 therapeutic agent presently available for the management of bleeding episodes; new approaches and treatments clearly are required.

KEY POINTS

- Hemophilia is an X-linked disorder resulting from deficiencies of FVIII or FIX and is categorized as mild, moderate, or severe depending on the factor level.
- Patients with severe hemophilia are at risk for developing joint disease, termed hemophilic arthropathy, which can be prevented by regular prophylactic factor infusions begun at an early age.
- Factor replacement therapy is available to treat bleeding episodes and is highly effective.
- Patients with hemophilia, most notably those with severe disease, may develop neutralizing antibodies directed against the deficient factor—termed inhibitors; inhibitors are divided into high- and low-responding types, and the presence of an inhibitor may render replacement therapy ineffective.
- Inhibitors can be eradicated with ITI in approximately 70% of patients with hemophilia A.
- Patients with high-responding inhibitors are infused with bypassing agents to treat or prevent bleeding episodes; overall, bypassing products are not as effective as standard factor replacement in noninhibitor patients, and as such, inhibitor patients have an increased risk of hemorrhage-associated morbidity and mortality.

Acquired hemophilia

Pathophysiology and etiology

Rarely, hemophilia can be acquired as a result of the development of autoantibodies most commonly directed against FVIII and is referred to as *acquired hemophilia*. It has been associated with a variety of conditions, including pregnancy, malignancies, and autoimmunity. In ~50% of cases, no known associated disorder can be identified. Overall, the annual incidence is 1.4 per million, though the frequency increases with age, with the median age of onset approximately 77 years. The anti-FVIII autoantibodies inhibit the functional activity of endogenous FVIII, resulting in a bleeding diathesis. Although some bleeding symptoms are similar to congenital hemophilia, the incidence of hemarthroses in acquired hemophilia is low, whereas soft tissue, abdominal, and retroperitoneal hemorrhage are more frequent. Additionally, bleeding in patients with acquired hemophilia may be more severe than is seen in congenital hemophilia, despite similar FVIII levels.

Clinical presentation

Acquired hemophilia may present with the dramatic onset of either mucocutaneous or internal bleeding. Life-threatening bleeding, such as gastrointestinal, intracranial,

or large muscle hematoma with associated compartment syndrome is observed. Hemarthroses are uncommon. In the era of bypass therapy, fatal bleeding is reported in 3% to 9% of patients.

Diagnosis

Acquired hemophilia should be suspected in patients that present with bleeding symptoms and a prolonged aPTT. Rarely, patients present with an asymptotically prolonged aPTT. Thus, evaluation of a prolonged aPTT in an adult should include a mixing study and FVIII level, regardless of the presence or absence of bleeding. Acquired FVIII inhibitors are typically time- and temperature-dependent, which translates into a normal immediate mixing study that then fails to correct with mixing after incubation.

Treatment

The management of bleeding episodes in acquired hemophilia is similar in many respects to that of congenital hemophilia with inhibitors, and the principles outlined earlier largely apply. An exception of note is that patients with acquired hemophilia often are elderly and at increased risk for thrombosis; thus, bypassing agents, although often required for control of bleeding, may have an associated higher rate of thrombotic complications. Recombinant porcine FVIII (Obizur; Baxalta, Westlake Village, CA) is available for treatment of bleeding in patients with acquired FVIII inhibitors. It was shown to be effective at reducing bleeding in 28 patients treated as part of a phase 2/3 study. The starting dose is 200 IU/kg, with subsequent dosing and frequency titrated based on FVIII levels. FVIII levels are recommended to be performed 30 minutes and 3 hours after the initial dose and 30 minutes after subsequent doses. Responses vary according to the amount of anti-FVIII inhibitor that is present and the degree to which it is cross-reactive with porcine FVIII. Strategies to promote inhibitor eradication in acquired hemophilia are different than in congenital hemophilia with inhibitor. Because acquired hemophilia is due to the development of autoantibodies that result from loss of self-tolerance, it tends to respond to immunosuppressive medications effective in autoimmune disorders in general. Corticosteroids are considered first-line therapy and should be used even in patients without current bleeding symptoms. Patients with detectable FVIII levels and inhibitor concentrations <20 BU/mL may respond to corticosteroids alone. Patients with inhibitor titers >20 BU/mL are less likely to respond to corticosteroids alone and cyclophosphamide should be added up front. The role of rituximab in up-front therapy remains controversial. Although rituximab registry data do not indicate that

rituximab increases the response rate, there is some evidence that it may reduce the rate of relapse. Since patients with acquired hemophilia continue to produce their own FVIII, exogenous administration, as is done with ITI, is not required for inhibitor eradication in this setting. Relapses occur in approximately 10% to 20% of patients, most often during the first year, and thus ongoing monitoring is essential.

Rare factor deficiencies

Deficiencies of other coagulation factors that play a role in thrombin generation, cross-linking, and stabilization of the fibrin clot or downregulation of fibrinolysis may lead to a bleeding diathesis. Deficiencies of fibrinogen, factor II (FII), FV, FVII, FX, and FXIII result in bleeding disorders in cases in which the severity of the bleeding is loosely related to the factor levels (Table 10-5). Although FVIII and FIX deficiency are defined as rare disorders affecting approximately 20,000 Americans, deficiencies of these other coagulation factors are far less common. Therefore,

the clinical presentation related to any specific level and the range of symptoms experienced are less well described than in hemophilia A and B. For detailed discussion of these disorders, see the special issue of the *British Journal of Haematology* (volume 167, issue 3, November 2014).

Fibrinogen deficiency

Pathophysiology

As discussed in the overview of hemostasis, fibrinogen is cleaved by thrombin to form fibrin, which then polymerizes to form tight thin strands in a meshwork that forms the clot (Figure 10-3).

Etiology

Congenital deficiencies of fibrinogen are due to defects in the genes (*FGA*, *FGB*, *FGG*) that code for 1 of 3 fibrinogen protein chains (Aa, Bb, and g) and can be inherited both in autosomal dominant or recessive patterns. Deficiencies can be complete (afibrinogenemia) or partial (hypofibrinogenemia) and associated with dysfunction

Table 10-5 Bleeding sites and symptoms and factor replacement choices for rare factor deficiencies

Factor deficiency (level associated with major bleeding)*	Bleeding sites	Other symptoms	Factor replacement	Acquired deficiencies
Fibrinogen (<10 mg/ dL)	No typical sites	Splenic rupture Miscarriage Thrombosis	Fibrinogen concentrate: RiaStap Cryoprecipitate	Liver disease Asparaginase therapy DIC
Factor II (<10%)	No typical sites	None	PCC	Vitamin K deficiency Liver disease Vitamin K antagonists Antiphospholipid syndrome
Factor V (<1%)	No typical sites	None	FFP platelet transfusion	Topical bovine thrombin exposure, antibiotics
Factor VII (<10%)	Intracranial	Thrombosis	rFVIIa	Vitamin K deficiency Liver disease Vitamin K antagonists
Factor X (<10%)	Intracranial	None	PCC	Vitamin K deficiency Liver disease Vitamin K antagonists Amyloidosis
Factor XI (no clear as- sociation between levels and bleeding)	Surgery or injury related	None	FFP FXI concentrates avail- able in some countries	Autoantibodies (rare)
Factor XIII (<5%)	Intracranial Umbilical stump	Poor wound healing Miscarriage	pdFXIII concentrate: Corifacit rFXIII: Tretten	Cardiopulmonary bypass Inflammatory bowel disease

RiaStap is licensed for congenital afibrinogenemia. Recombinant factor VIIa is licensed for the treatment of congenital FVII deficiency. Corifacit and Tretten are licensed for congenital FXIII deficiency. Prothrombin complex concentrates (PCC) not licensed for the treatment of rare factor deficiencies and contain variable amounts of factors II, VII, and X, with dosing based on FIX units.

DIC, disseminated intravascular coagulation; FFP, fresh frozen plasma; PCC, prothrombin complex concentrate.

*Official Communication of the Scientific Subcommittee on Rare Bleeding Disorders of the ISTH.

(hypodysfibrinogenemia). Acquired causes of hypofibrinogenemia include liver disease, use of chemotherapeutic agents such as L-asparaginase, and the Kasabach-Merritt syndrome (hemangioma with consumptive coagulopathy). Other consumptive processes, such as disseminated intravascular coagulation (DIC), lead to multiple coagulation factor deficiencies in addition to fibrinogen.

Clinical presentation

Bleeding can be variable, with potential sites being mucocutaneous, soft tissue, intracranial, umbilical stump traumatic, postsurgical bleeding, and recurrent miscarriages. Particularly in the setting of a dysfibrinogenemia and hypodysfibrinogenemia, thrombosis may be coexistent with bleeding. Although patients with severe symptoms are more likely to have lower levels, the levels of those that are symptomatic and asymptomatic overlap.

Diagnosis

Diagnosis is suspected in the setting of both a prolonged PT and aPTT. The thrombin time is also prolonged and fibrinogen activity reduced. Once a low fibrinogen activity is measured, a fibrinogen antigen should be measured. Afibrinogenemia and hypofibrinogenemia have similar fibrinogen activity and antigen levels, whereas patients with dysfibrinogenemia have higher antigen than activity levels. Molecular testing can be performed to confirm that diagnosis, but it is not routinely available.

Treatment

Fibrinogen concentrate is approved for treatment of fibrinogen deficiency (afibrinogenemia and hypofibrinogenemia). For treatment of bleeding or prior to invasive procedures, the dose of fibrinogen concentrate can be calculated as (target – baseline)/1.7 × weight in kg. The half-life of fibrinogen is approximately 80 hours, thus repeat dosing can be given every 2 to 4 days as needed to maintain a fibrinogen level of >100 to 150 mg/dL. Cryoprecipitate can be used if a fibrinogen concentrate is not available (1 unit per 5 to 10 kg of body weight).

Prothrombin deficiency

Pathophysiology

As discussed in the overview of hemostasis, a small amount of thrombin is produced by the TF:FVIIa complex and is needed for initiation of coagulation. A large burst of thrombin is produced by cleavage of prothrombin to thrombin by the thrombinase complex on the activated platelet surface. Thrombin has both pro- and anticoagulant functions. Thrombin's main procoagulant function is to cleave fibrinogen to fibrin.

Etiology

Both quantitative and qualitative defects of FII can be inherited as an autosomal disorder and its prevalence is estimated to be 1 in 2,000,000. Acquired deficiencies of FII can occur in association with antiphospholipid syndrome as a result of antiprothrombin antibodies that lead to increased prothrombin clearance (hypoprothrombinemia–lupus anticoagulant syndrome), liver disease, or vitamin K deficiency.

Clinical presentation

There is a poor correlation between clinical symptoms and FII levels. Bleeding symptoms are reported more commonly with FII levels <10% and may include mucocutaneous, soft tissue, joint, surgical, and menstrual bleeding. Heterozygous carriers typically are asymptomatic.

Diagnosis

The diagnosis should be considered in the setting of a prolonged PT and aPTT with a normal thrombin time (deficiencies of FV and FX have a similar pattern). FII activity can be measured using a 1-stage PT-based assay. The presence of antiphospholipid antibodies, liver failure, and vitamin K deficiency should also be evaluated when FII deficiency is suspected.

Treatment

Management of bleeding in patients with FII deficiency is with prothrombin complex concentrates (PCC); 20 to 30 IU/kg is expected to increase the plasma FII concentration by 40% to 60%. Since FII's half-life is 60 hours, doses of PCC can be repeated every 2 to 3 days as needed.

Factor V deficiency

Pathophysiology

Factor V acts as a cofactor for FX to potentiate FXa cleavage of prothrombin.

Etiology

Deficiency of FV is an autosomal disorder and is estimated to have a prevalence of 1 in 1,000,000. FV deficiency can also occur in combination with FVIII deficiency. Combined FV and FVIII deficiency is the result of mutations in 2 genes, *LMAN1* and *MCFD2*. These genes encode for proteins that participate in transporting FV and FVIII from the ER to the Golgi necessary for normal secretion of FV and FVIII into the circulation. Very rarely, acquired FV deficiency can occur after exposure to bovine thrombin found in topical thrombin preparations and results

in formation of antibodies against bovine thrombin that cross-react with human FV.

Clinical presentation

Bleeding symptoms are diverse, with the most severe bleeding occurring in those with levels <1%, though there is overlap of FV levels between those that are symptomatic and those that are asymptomatic.

Diagnosis

The laboratory investigation demonstrates a prolonged PT and aPTT and a normal thrombin time. Other common pathway clotting factors (fibrinogen, FII, and FX) are normal, and FV activity using a 1-stage PT-based assay is low. FVIII activity should also be measured to differentiate between combined FV and FVIII deficiency and isolated FV deficiency.

Treatment

There is currently no FV concentrate available, thus replacement of FV at the time of bleeding or prior to invasive procedures requires infusion of fresh frozen plasma (FFP). Additionally, platelet α -granules contain 20% of circulating FV; thus, platelets can be a source of FV and used in combination with FFP when FFP alone has been ineffective. In general, 15 mL/kg of FFP is estimated to raise the FV activity 15%. The half-life of FV following FFP infusion is 16 to 36 hours.

Factor VII deficiency

Pathophysiology

Factor VII is a vitamin K-dependent protein, and 1% circulates in the active form (FVIIa) available to bind exposed TF at sites of vascular injury.

Etiology

FVII deficiency is an autosomal recessive disorder with an estimated worldwide prevalence of 1 in 500,000.

Clinical presentation

Patients with severe FVII deficiency, FVII <1%, are those most likely to have significant bleeding, including intracranial hemorrhage. In an international registry, cases with severe bleeding had levels of 0% to 21%, and those that were asymptomatic had levels of 15% to 35%.

Diagnosis

The laboratory picture of FVII deficiency is that of an isolated prolonged PT with normal aPTT and thrombin time. An isolated prolonged PT should prompt measure-

ment of FVII activity. Early vitamin K deficiency may predominantly affect the FVII activity.

Treatment

Treatment of FVII deficiency is with rFVIIa (15 to 20 μ g/kg) with doses repeated every 6 hours as needed. Antifibrinolytics (EACA or TXA) may also be useful for minor bleeding, particularly in those with no or minimal personal history of bleeding. FVII levels of >10% to 20% are typically adequate for surgery. In the setting of severe deficiency and recurrent bleeding, prophylaxis with rFVIIa (20 to 30 μ g/kg) 2 to 3 times weekly has been reported to be effective at preventing bleeding events.

Factor X deficiency

Pathophysiology

Factor Xa, in conjunction with its cofactor, FVa, cleaves prothrombin for thrombin.

Etiology

Congenital FX deficiency is an autosomal recessive disorder with an estimated worldwide prevalence of 1 in 1,000,000. Acquired FX deficiency can be seen in the setting of AL amyloidosis secondary to binding of FX to AL amyloid, effectively removing it from circulation.

Clinical presentation

Abnormal bleeding in patients with FX deficiency can manifest as mucocutaneous, soft tissue, or gastrointestinal bleeding. Importantly, intracranial bleeding was reported in up to 21% of symptomatic cases. Severe bleeding symptoms are more likely to occur in the setting of FX activity <10%.

Diagnosis

Laboratory testing in patients with FX deficiency show a prolonged PT and aPTT, but normal thrombin time similar to FII and FV deficiency. FX activity is typically measured using a 1-stage PT-based assay. The choice of thromboplastin used in the assay may influence the FX activity result. Congenital FX deficiency is distinguished from acquired FX deficiency secondary to AL amyloidosis on clinical grounds. Mixing studies in the setting of AL amyloidosis demonstrate correction and appear consistent with a deficiency.

Treatment

Treatment of FX deficiency is with PCC (FX concentrates may be available in some countries). A typical dose of PCC is 20 to 30 IU/kg and would be expected to increase the plasma FX activity 40% to 60%.

The half-life of FX is approximately 30 hours, and thus repeat doses of PCC can be given every 1 to 2 days as needed to maintain hemostasis. In the setting of AL amyloidosis, treatment of the AL amyloid typically normalizes the FX level. If hemostatic support is needed prior to treatment of the underlying AL amyloidosis, as may occur with emergent surgery, PCC (20 to 30 IU/kg) can also be used, though strong evidence to support treatment decisions is lacking.

Factor XI deficiency

Pathophysiology

Factor XI is activated by thrombin after initiation of coagulation. FXIa is then available to activate FIX on the activated platelet surface, providing an amplification loop. FIX activation by FXIa is required for generation of a burst of thrombin that is adequate to activate TAFI. In the absence of activated FXI to promote an adequate burst of thrombin, clots are more susceptible to fibrinolysis.

Etiology

FXI deficiency can occur as both autosomal dominant and recessive. Prevalence has been difficult to estimate due to variability of the clinical phenotype, but it is known to be higher in Jewish populations, where 1 in 11 are heterozygous and 1 in 450 are homozygous or compound heterozygous. FXI is activated during the initiation phase by thrombin on the platelet surface.

Clinical presentation

Bleeding after surgery or trauma is the most common manifestation of FXI deficiency, as well as in sites where fibrinolysis is active, such as the gastrointestinal tract and urogenital system. Spontaneous bleeding is uncommon. The clinical phenotype is quite variable, and there is a weak correlation between FXI level and bleeding.

Diagnosis

Laboratory testing in the setting of FXI deficiency demonstrates a prolonged aPTT and normal PT and thrombin time. FXI activity is measured using a 1-stage aPTT assay.

Treatment

Since the FXI level is such a poor predictor of bleeding, the presence or absence of bleeding with prior traumatic events or invasive procedures should be considered when determining bleeding risk and need for treatment. Antifibrinolytic therapy with TXA or EACA should be a mainstay of treatment. For persons with FXI levels <10% or with a personal history of bleeding, replacement of FXI

using FFP (15 to 25 mL/kg) can be considered for severe bleeds or major surgery. FXI concentrate is available in some countries, but not in the United States. Alloantibodies against FXI (FXI inhibitor) have been reported to occur following replacement therapy.

Factor XIII deficiency

Pathophysiology

FXIII circulates as a heterotetramer with 2 catalytic A subunits and 2 carrier B subunits. FXIII is activated by thrombin and once activated covalently crosslinks fibrin.

Etiology

Congenital factor XIII deficiency is an autosomal recessive disorder with a worldwide prevalence estimated to be 1 in 2,000,000. Acquired FXIII deficiency can occur in the setting of cardiac surgery, malignancy, infection, and inflammatory bowel disease. FXIII can be caused by mutations in the genes that code for either the catalytic A subunit or the B carrier subunit, though mutations in subunit B are reported to account for <5% of cases of congenital factor XIII deficiency.

Clinical presentation

The clinical phenotype is similar regardless of the subunit affected. The most common sites of bleeding are umbilical stump, soft tissue, surgical, and intracranial hemorrhage. In addition to bleeding, poor wound healing is often present and pregnancy loss can occur. Heterozygous carriers may have FXIII activity levels as low as 20% and may display mild bleeding symptoms.

Diagnosis

Laboratory diagnosis requires measurement of FXIII activity because the results of typical screening tests such as PT, aPTT, and thrombin time are normal. Qualitative assays for FXIII activity (clot solubility) are only abnormal with levels <5%. Quantitative assays are also available and can detect abnormal FXIII levels despite a normal clot solubility test. Genetic analysis is the most effective means to determine if subunit A or B is affected.

Treatment

Given the high rate of intracranial hemorrhage, prophylaxis with FXIII concentrate is recommended in all patients with a FXIII level <10%. Since FXIII has a half-life of 7 days, hemostatic FXIII levels can be maintained by administering FXIII concentrates every 28 days. Available concentrates include plasma-derived FXIII (pd-FXIII) (Corifact; CSL Behring) and a recombinant FXIII

(rFXIII) (Tretten; Novo Nordisk). pdFXIII contains both subunits, whereas rFXIII contains only subunit A. Accordingly, patients with subunit B deficiency should be treated with pdFXIII and not rFXIII.

Vitamin K-dependent coagulation factor deficiency

Pathophysiology

Factors II, VII, IX, and X are vitamin K-dependent clotting factors. During synthesis, they undergo γ -glutamyl carboxylation by γ -glutamyl carboxylase and the co-factor vitamin K hydroxyquinone (KH_2). During γ -carboxylation, KH_2 is oxidized to vitamin K 2,3-epoxide, which then undergoes de-epoxidation by vitamin K oxide reductase (VKOR) to restore KH_2 .

Etiology

Vitamin K-dependent coagulation factor deficiency (VKDCFD) is an autosomal recessive disorder that has been reported to occur in fewer than 30 families worldwide. It is caused by a defect in the γ -glutamyl carboxylase protein or in subunit 1 of VKOR protein and leads to deficiencies of vitamin K-dependent clotting factors: FII, FVII, FIX, and FX.

Clinical presentation

Clinically, VKDCFD presents at birth with intracranial or umbilical bleeding or early childhood with joint, mucocutaneous, or soft-tissue bleeding.

Diagnosis

Factors II, VII, IX, and X are reduced. Distinguishing VKDCFD from acquired vitamin K deficiency requires demonstration of a normal fasting KH_2 concentration.

Treatment

Treatment with oral or parenteral vitamin K1 has been shown to partially or completely restore coagulation factor activities and is the mainstay of long-term therapy for prevention of bleeding.

Gaps in knowledge

Large, well-designed prospective studies of congenital rare factor deficiencies are not possible due to the low disease prevalence. Much of current knowledge of these conditions is derived from registry data and small interventional studies. There is a need for both epidemiologic and therapeutic studies in these disorders. Development of international databases is required to establish the natural history and treatment outcomes of these disorders from which minimally active hemostatic levels can be established.

A major limitation in some of these conditions is the lack of availability of a specific replacement concentrate for treatment. Presently in the United States, 3 licensed products for rare disorders are available, specifically for afibrinogenemia, FVII, and FXIII deficiency. A specific concentrate for FXI deficiency is available in the European Union. In the United States, off-label use of products continues, including use of PCC for deficiencies of FX and FII. In FV and FXI deficiency, FFP remains the mainstay of therapy; in addition, platelet transfusions are sometimes used in FV deficiency because platelets also contain FV. Even when a concentrate is available, its use in these rare disorders often is guided by personal experience or anecdotal reports. For example, determination of appropriate patients for whom prophylaxis is indicated and the appropriate dosing regimen is largely poorly defined. Also, the peri- and postoperative care of patients with rare disorders is not founded on evidence-based data. There is a clear need for consistent data collection and studies on the clinical management of rare factor deficiencies.

KEY POINTS

- Rare factor deficiencies occur as a result of genetic mutations and acquired disorders.
- Treatment of an associated underlying disorder may lead to the resolution of the acquired deficiency.
- Rare factor deficiencies result in highly variable bleeding symptoms, ranging from injury or interventional bleeding (FXI) to severe spontaneous intracranial bleeding (FX and FXIII).
- Few specific factor replacement concentrates are available for patients with rare factor deficiencies.

Disorders of fibrinolysis

Pathophysiology

The fibrinolytic system provides orderly clot remodeling and dissolution. Imbalances in fibrinolysis may lead to excessive fibrinolytic activity through a variety of mechanisms, including increased tPA activity or inadequate inhibition as the result of PAI-1 or α_2 AP deficiencies, and may result in excessive bleeding.

Etiology

Hyperfibrinolysis may result from congenital deficiencies of PAI-1 or α_2 AP. PAI-1 deficiency is extraordinarily rare, and in only a few cases has the genetic alteration causing the disorder been identified. Defects in α_2 AP also have been described. Both conditions are inherited as autosomal

recessive traits. Additionally, hyperfibrinolysis may occur due to a variety of acquired conditions, including liver disease and DIC; after surgery, particularly cardiac surgery; and some prostatic diseases and cases of acute promyelocytic leukemia. Although these conditions also contribute to bleeding for other reasons (factor deficiencies due to liver disease, consumption of clotting factors in DIC, and platelet dysfunction in cardiac surgery), the possibility of a contributing hyperfibrinolytic state should be considered, as specific therapies are available.

Clinical presentation

The clinical presentation of hyperfibrinolysis is highly variable. Hyperfibrinolytic bleeding may occur in isolation as a result of a congenital deficiency; but most commonly, it occurs as a part of a complex coagulopathy in an acquired disorder. Congenital deficiencies of the fibrinolytic pathway may present with delayed bleeding after injury or intervention and may include mucus membrane, cutaneous, or deep tissue bleeding; however, intracranial hemorrhage has been reported in PAI-1 and α_2 AP deficiency. Acquired hyperfibrinolysis presents with bleeding at a variety of sites, and in patients with recent surgery, delayed postoperative hemorrhage often occurs at the surgical site.

Diagnosis

Laboratory investigation of the fibrinolytic system is difficult. The euglobulin clot lysis time (ELT) currently is not available in all laboratories, and interpretation of results is not always straightforward. The ELT assesses the capacity of plasma to lyse a clot formed in patient plasma. Under assay conditions, a clot is expected to dissolve within a set period of time, commonly approximately 2 to 6 hours, and a shortened ELT suggests hyperfibrinolysis. Several new global hemostatic assays are under evaluation for their ability to more accurately detect hyperfibrinolysis. A currently available global assay is the thromboelastogram and most commonly is used in surgical settings; thromboelastography is a method to assess global hemostasis and can detect hyperfibrinolysis in cases in which the use of antifibrinolytic agents may be helpful to control excessive bleeding.

It is possible to measure a few individual components of the fibrinolytic system, including α_2 AP and plasminogen. Although it is possible to measure antigenic levels of PAI-1, the activity assay is problematic because the normal range includes levels of zero, thereby making detection of a dysproteinemic deficiency state impossible. Elevated PAI-1 levels have been associated with atherosclerosis and are not associated with bleeding. PAI-1 levels also exhibit

diurnal variation, and any one level may not represent either the highest or lowest physiologic level. A deficiency of α_2 AP is measurable; however, the correlation of level of deficiency and risk for bleeding is poorly established. It also is possible to measure the fibrinolytic proteins tPA and plasminogen, with a hyperfibrinolytic state expected to result in increased tPA and decreased plasminogen. Again, the correlation between specific levels and the degree of hyperfibrinolysis has not been established.

Therefore, laboratory diagnosis of the fibrinolytic system presently is not optimal, requiring the clinician to rely on clinical suspicion, including the presence of delayed bleeding, the clinical context and, at times, response to therapeutic interventions.

Treatment

The treatment of hyperfibrinolytic bleeding is fairly straightforward except when it occurs as a complex coagulopathy, when treatment requires careful consideration of thrombotic risk. The control of fibrinolytic bleeding is based on the use of antifibrinolytic agents; although several agents are available, 2 are most widely used: EACA and TXA. The mechanism of action of both agents involves competition with negatively charged lysine-rich residues in the kringle domain of plasminogen, which render plasminogen resistant to activation by tPA. Thus, these agents are effective in tissues rich in tPA. Both are available for intravenous and oral administration. Adverse effects and precautions were described previously. When using antifibrinolytic therapy, it is important not to discontinue therapy prematurely because of the risk of delayed bleeding. It is recommended to continue therapy up until the hyperfibrinolysis is felt to have resolved, or possibly on an ongoing basis if a congenital defect is confirmed and ongoing therapy is warranted.

Prognosis and outcomes

Most commonly encountered causes of hyperfibrinolysis are acquired; with trigger resolution, the patient's hemostatic system should return to normal and, provided that catastrophic bleeding has not occurred, patients should recover without sequelae. For the rare patient with a confirmed congenital disorder, management with antifibrinolytic agents, even as prophylaxis, can minimize or reduce bleeding symptoms.

Gaps in knowledge

The major gap in knowledge in these conditions is the ability to establish an accurate diagnosis because treatment is less difficult than diagnosis. The fibrinolytic pathway

remains the most problematic both in terms of diagnosis of a deficiency state and clearly attributable clinical manifestations. Improved and specific laboratory methods are required. A reliable, easily performed, reproducible screening assay would represent an important first step in the diagnosis of these disorders, followed by development of specific factor assays for all components of the fibrinolytic system. Levels of deficiency correlated with clinical bleeding could then be established. An improved understanding of the genetics of congenital fibrinolytic deficiencies and the associated spectrum of clinical manifestations would assist clinicians in the diagnosis of these rare disorders.

KEY POINTS

- Fibrinolytic disorders are the least well-defined hemorrhagic diatheses.
- Hyperfibrinolytic disorders should be suspected in the setting of delayed bleeding.
- Hyperfibrinolytic disorders are most often acquired, although rare congenital defects have been documented.
- Laboratory diagnosis of fibrinolytic disorders is difficult and inconsistently precise.
- Treatment of hyperfibrinolytic bleeding is based on the use of antifibrinolytic agents, including EACA and TXA.

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11

Disorders of platelet number and function

MICHELE LAMBERT, ADAM CUKER,
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The online version of this chapter contains an educational multimedia component on platelet function in health and disease.

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Off-label drug use: Desmopressin for inherited platelet function defects and uremic platelets. Recombinant VIIa for inherited platelet function defects. Rituximab for ITP and TTP. Fondaparinux, bivalirudin, and direct oral anticoagulants for HIT.

Platelet biology: structure and function

Hemostasis encompasses a series of interrelated and simultaneously occurring events involving the blood vessels, platelets, coagulation system, and the fibrinolytic pathway. Defects affecting any of these major participants may lead to a hemostatic defect and a bleeding disorder. This chapter focuses on disorders related to platelet number and function.

Platelet structure

Blood platelets are anucleate fragments derived from bone marrow megakaryocytes. The platelet diameter ranges from 1.5 to 3.0 μm , roughly one-third to one-fourth that of an erythrocyte. Mean platelet volume is approximately 7 fL. Electron microscopy reveals a fuzzy coat (glycocalyx) on the platelet surface composed of membrane glycoproteins (GPs), glycolipids, mucopolysaccharides, and adsorbed plasma proteins. The plasma membrane is a bilayer of phospholipids in which cholesterol, glycolipids, and GPs are embedded. The phospholipids are asymmetrically organized in the plasma membrane; negatively charged phospholipids (such as phosphatidylserine [PS]) are present almost exclusively in the inner leaflet, whereas the others are more evenly distributed. Platelets have an elaborate channel system, the open canalicular system, which is composed of invaginations of the plasma membrane and extends throughout the platelet and opens to the surface. The discoid shape of the resting platelet is maintained by a well-defined cytoskeleton consisting of the spectrin membrane skeleton, the marginal microtubule coil, and the actin cytoskeleton. The microtubule coil, present below the platelet membrane, is made up of α - β -tubulin dimers and, together with nonmuscular myosin IIA, plays a role in platelet formation from megakaryocytes, in addition to maintaining the discoid platelet shape. In proximity to the open canalicular system is the dense tubular system, a closed-channel network derived from the smooth endoplasmic reticulum. It is considered the major site of platelet prostaglandin and thromboxane synthesis.

Platelets contain a variety of organelles: mitochondria and glycogen stores, lysosomes, peroxisomes, dense granules, and α -granules. The lysosomes contain acid hydrolases; the dense granules contain calcium (which gives them high electron density), adenosine triphosphate (ATP), adenosine diphosphate (ADP), magnesium, serotonin (5-hydroxytryptamine), and polyphosphates (which promote

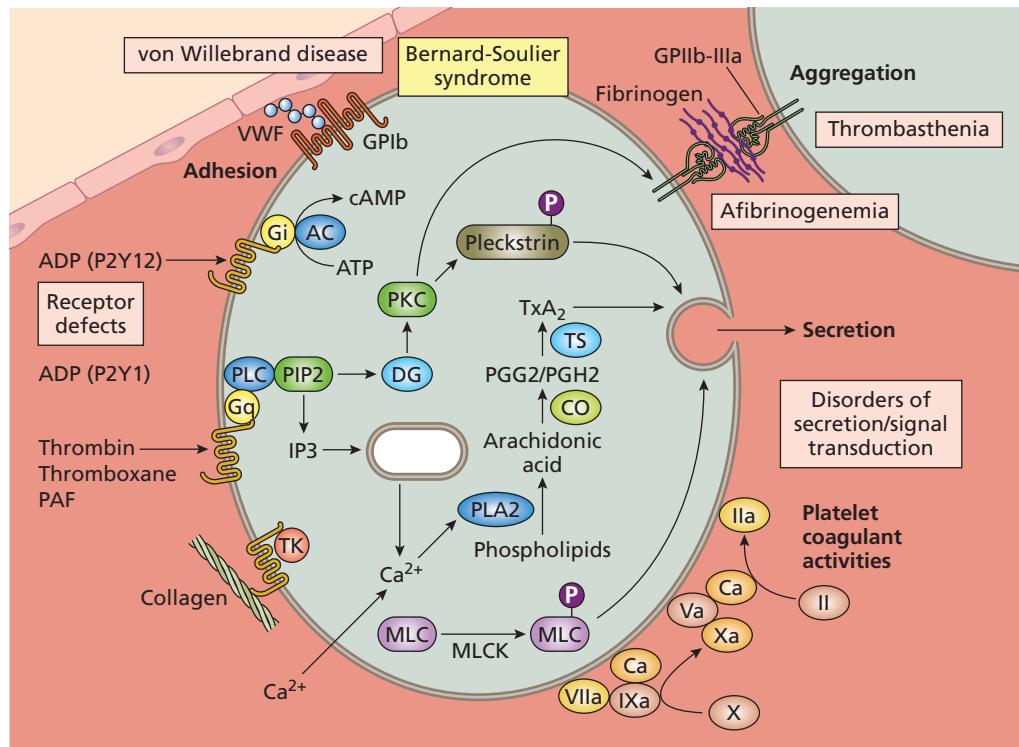
coagulation through various means, including activation of the intrinsic pathway). Serotonin is taken up by platelets from plasma and incorporated into the dense granules. The α -granules contain a large number of proteins, including β -thromboglobulin (β TG) and platelet factor 4 (PF4), which are considered platelet specific; several coagulation factors (eg, fibrinogen, factor V, factor XIII); von Willebrand factor (VWF); growth factors (eg, platelet-derived growth factor, vascular endothelial growth factor); vitronectin; fibronectin; thrombospondin; the factor V binding protein multimerin; P-selectin; albumin; and immunoglobulin G (IgG). Some of these (eg, VWF, PF4, β TG) are synthesized by megakaryocytes, whereas others (eg, albumin, IgG) are incorporated into the α -granules from plasma.

Platelet function in hemostasis

Following injury to the blood vessel (see video in online edition), platelets interact with collagen fibrils in the exposed subendothelium by a process (adhesion) that involves, among other events, the interaction of a plasma protein

(VWF) and a specific GP complex on the platelet surface, GP Ib-IX-V (GP Ib-IX) (Figure 11-1). This interaction is particularly important for platelet adhesion under conditions of high shear stress. After adherence to the vessel wall via VWF and the long GP Ib-IX-V receptor, other platelet receptors interact with proteins in the subendothelial matrix. Hereby, collagen provides not only a surface for adhesion but also serves as a strong stimulus for platelet activation. Activated platelets release the contents of their granules (secretion), including ADP and serotonin from the dense granules, which induces recruitment of additional platelets. These additional platelets form clumps at the site of vessel injury, a process called aggregation (cohesion). Aggregation involves binding of fibrinogen to specific platelet surface receptors, a complex composed of GPIIb-IIIa (integrin α IIb β 3). GPIIb-IIIa is platelet specific and has the ability to bind VWF as well. Although resting platelets do not bind fibrinogen, platelet activation induces a conformational change in the GPIIb-IIIa complex that leads to fibrinogen binding. Moreover, platelets play a major role in coagulation mechanisms. Several key

Figure 11-1 Schematic representation of selected platelet responses to activation and inherited disorders of platelet function. Roman numerals in circles represent coagulation factors. Modified with permission from Rao AK. *Am J Med Sci*. 1998;316:69–76. AC, adenylyl cyclase; CO, cyclooxygenase; DAG, diacylglycerol; G, guanosine triphosphate–binding protein; IP3, inositol trisphosphate; MLC, myosin light chain; MLCK, myosin light chain kinase; PAF, platelet-activating factor; PIP2, phosphatidylinositol bisphosphate; PLC, phospholipase C; PLA2, phospholipase A₂; TK, tyrosine kinase; TS, thromboxane synthase.



enzymatic reactions occur on the platelet membrane lipoprotein surface. During platelet activation, the negatively charged phospholipids, especially PS, become exposed on the platelet surface, an essential step for accelerating specific coagulation reactions by promoting the binding of coagulation factors involved in thrombin generation (platelet procoagulant activity).

A number of physiologic agonists interact with specific receptors on the platelet surface to induce responses, including a change in platelet shape from discoid to spherical (shape change), aggregation, secretion, and thromboxane A₂ (TxA₂) production. Other agonists such as prostacyclin inhibit these responses. Binding of agonists to platelet receptors initiates the production or release of several intracellular messenger molecules, including products of hydrolysis of phosphoinositide by phospholipase C (diacylglycerol and inositol 1,4,5-triphosphate [InsP₃]), TxA₂, and cyclic nucleotides (cyclic adenosine monophosphate) (Figure 11-1). These induce or modulate the various platelet responses of Ca²⁺ mobilization, protein phosphorylation, aggregation, secretion, and thromboxane production. The interaction between the platelet surface receptors and the key intracellular enzymes (eg, phospholipases A₂ and C, adenylyl cyclase) is mediated by a group of proteins that bind and are modulated by guanosine triphosphate (G proteins). As in most secretory cells, platelet activation results in an increase in cytoplasmic ionized calcium concentration; InsP₃ functions as a messenger to mobilize Ca²⁺ from intracellular stores. Diacylglycerol activates protein kinase C (PKC), resulting in phosphorylation of several proteins. PKC activation is considered to play a major role in platelet secretion and in the activation of GPIIb-IIIa. Numerous other mechanisms, such as activation of tyrosine kinases and phosphatases, are also triggered by platelet activation. Either inherited or acquired defects in these platelet mechanisms may lead to impairment of platelet function in hemostasis.

Regulation of platelet number

Overview

The platelet count is regulated by the relative rates of platelet production and clearance. Kinetic studies have demonstrated that the average platelet life span is 7 to 10 days. Platelets that are lost through senescence, activation, or other processes are replaced by new platelets derived from bone marrow megakaryocytes. Platelet production from megakaryocytes, in turn, is driven by the hormone thrombopoietin (TPO) and its cellular receptor, c-Mpl.

Thrombopoietin and the thrombopoietin receptor c-Mpl

A healthy adult produces 1×10^{11} to 3×10^{11} platelets per day, although production can increase 10-fold during times of high demand. The number of circulating platelets is regulated chiefly by TPO, which binds to megakaryocytes and hematopoietic stem cells via c-Mpl, which is a member of the class I hematopoietic growth factor receptor superfamily and activates several signaling pathways in megakaryocytes, resulting in megakaryocyte proliferation and differentiation, ultimately resulting in platelet production. c-Mpl is also expressed on mature platelets, which bind and clear TPO from the circulation. TPO is secreted constitutively from the liver; although its synthesis may increase slightly during thrombocytopenic states, its overall production is relatively constant. As a consequence, the level of free TPO is regulated primarily by the number of circulating platelets, the platelet life span, and the megakaryocyte mass. Recent mouse studies have challenged this paradigm. The Ashwell-Morell receptor on murine hepatocytes binds platelets that have lost sialic acid residues on their surface. Binding activates a JAK-STAT signaling pathway, resulting in increased hepatic TPO mRNA and TPO production. The relevance of this pathway to normal human thrombopoiesis is not yet known.

In conditions such as aplastic anemia, which is characterized by a low platelet count and decreased bone marrow megakaryocyte mass, free TPO levels are high. In immune thrombocytopenia, the megakaryocyte mass may be expanded and platelet clearance is accelerated. This results in enhanced TPO clearance and plasma TPO levels that usually fall within the normal range, despite thrombocytopenia. The role of TPO as the principal physiologic regulator of platelet production has been confirmed in studies of TPO and c-Mpl deficient mice, which have 5% to 15% of normal levels of circulating platelets, megakaryocytes, and megakaryocyte progenitor cells. TPO alone, however, does not fully support megakaryocyte polyploidization *in vitro*, suggesting that additional factors—such as stem cell factor, interleukin 3, interleukin 6, and interleukin 11—are required for optimal megakaryocyte development.

Normal platelet production

Megakaryocyte proliferation and differentiation involve endomitosis and polyploidization, a process in which the nucleus divides but the cell does not. In the process of maturation, megakaryocytes form secretory granules and a demarcation membrane system that permeates the cytoplasmic space. This extensive membrane system eventually projects multiple filamentous pseudopodial

structures called proplatelets. This process utilizes the entire repertoire of cytoplasmic granules, macromolecules, and membranes. Ultimately, fragmentation of the pseudopodial projections leads to the release of new platelets. The exact steps leading from megakaryocytes to mature platelets are still not fully resolved. Different mechanisms are proposed and have been shown in mouse models: (1) at the sinusoids of the bone marrow the megakaryocytes produce long proplatelet strings, from which individual platelets rupture; (2) larger fragments of megakaryocytes consisting of proplatelets are released into the sinusoids, which then divide into individual platelets in the circulation; and (3) the entire megakaryocyte migrates into the sinusoids, is transported in the bloodstream into the lung, where the shear forces in the lung arterioles cause release of platelets. It is likely that all 3 models are at least partially true and together contribute to platelet production from megakaryocytes. Each megakaryocyte produces 1,000 to 3,000 platelets before the remaining nuclear material is phagocytosed by resident macrophages. Released platelets circulate for 7 to 10 days before undergoing senescence and clearance by phagocytic cells in the reticuloendothelial system.

KEY POINTS

- The primary mediator of platelet production is TPO, produced primarily by the liver.
- TPO production is largely constitutive; TPO levels are regulated by the platelet and megakaryocyte mass through binding of TPO to its receptor, c-Mpl.
- TPO levels are typically normal in immune thrombocytopenia (ITP) (representing relative deficiency compared to platelet count), but are elevated in bone marrow failure syndromes.
- The normal platelet life span is 7 to 10 days.

Immune causes of thrombocytopenia

CLINICAL CASE

A 68-year-old man is referred for evaluation of increased bruising, primarily on his forearms, for the last 3 months. He restores old cars as a hobby and believes that trauma associated with this work may have caused his bruises, although he cannot recall specific instances during which he injured himself. He denies epistaxis, melena, or other evidence of systemic bleeding. His medical history is otherwise notable

for mild hypertension treated with an angiotensin-converting enzyme inhibitor. He does not take other prescription medications but takes fish oil and vitamin C supplements. On physical examination, he appears well. Several 2.0-cm bruises are noted on the distal upper extremities and backs of the hands. Complete blood count reveals a hemoglobin of 13.8 g/dL, white blood cell (WBC) count of $6.9 \times 10^9/L$, and platelet count of $22 \times 10^9/L$.

Immune thrombocytopenia

ITP is an autoimmune disorder characterized by thrombocytopenia and a variable risk of bleeding. An international working group proposed standard terminology and definitions for ITP. The term *immune* is now used instead of *idiopathic* and the term *purpura* has been abandoned because bleeding symptoms, including purpura, are not present in all cases. Thus, the working group recommended the term *immune thrombocytopenia*, although the abbreviation ITP is preserved. In the working group's classification scheme, *primary* is used to denote ITP with no apparent precipitating cause, while *secondary* ITP refers to immune-mediated thrombocytopenia in which a predisposing condition can be identified. ITP is also classified according to disease duration. Within 3 months of presentation, ITP is termed newly diagnosed. ITP lasting 3 to 12 months and >12 months is denoted as *persistent* and *chronic*, respectively (Table 11-1). This terminology was adopted in the ITP guideline developed by the American Society of Hematology (ASH).

ITP is a relatively common cause of thrombocytopenia in adults and children. Estimates of prevalence vary, ranging between 3 and 20 per 100,000 persons, with an estimated incidence of 2 to 10 cases per 100,000 patient-years. In childhood, the highest incidence is in children <5 years old, with a gradual decrease toward adolescence. Most studies find the incidence to be equal in girls and boys, although some reports suggest a higher incidence in boys <5 years old. In adults, the incidence and prevalence of ITP is greatest in the elderly, with a female preponderance in the middle-adult years and a slight male preponderance in patients >70 years of age. In children, ITP often occurs after an antecedent viral infection and is self-limited in 80% of cases. In contrast, primary ITP assumes a chronic course in approximately 75% of adult patients. Although patients with more severe thrombocytopenia may present with mucocutaneous bleeding, those diagnosed with thrombocytopenia on a routine blood count are often asymptomatic. There is no gold-standard laboratory test for ITP. Although detection of GP-specific antiplatelet antibodies on the patient's platelets suggests the diagnosis,

Table 11-1 ITP definitions

Primary ITP	<ul style="list-style-type: none"> • Isolated thrombocytopenia • Platelets $<100 \times 10^9/L$ • No other apparent causes of thrombocytopenia • No secondary cause of ITP present
Secondary ITP	<ul style="list-style-type: none"> • All other forms of immune-mediated thrombocytopenia except primary ITP • Designate with presumed cause, in parentheses (eg, lupus-associated)
Phases of ITP	<ul style="list-style-type: none"> • Newly diagnosed: within 3 months of diagnosis • Persistent: between 3 and 12 months of diagnosis • Chronic: lasting >12 months

Adapted from Rodeghiero F et al. *Blood*. 2009;113:2386–2393.

these antibodies are detectable in only about 60% of patients with ITP. Moreover, antiplatelet antibodies may be detected in thrombocytopenic patients without ITP (eg, in microangiopathies in which damaged platelets expose immunogenic epitopes). The diagnosis of ITP is primarily made by excluding nonimmune causes of thrombocytopenia and investigating potential secondary causes. The most compelling evidence supporting a diagnosis of ITP is a platelet response to ITP-specific therapy.

Secondary ITP occurs in the setting of lymphoproliferative disorders; systemic lupus erythematosus, antiphospholipid syndrome, or other autoimmune disorders; infections such as hepatitis C, HIV, and *Helicobacter pylori*; and immune deficiency states such as common variable immune deficiency. Drug-induced immune thrombocytopenia is described in the section “Drug-induced immune thrombocytopenia” later in this chapter. Nonimmune causes of thrombocytopenia including hypersplenism, hereditary thrombocytopenias, thrombotic thrombocytopenic purpura (TTP), and type 2B von Willebrand disease (VWD) should be included in the differential diagnosis of ITP. Occasional patients with myelodysplastic syndromes or aplastic anemia may present with isolated thrombocytopenia.

Clinical features of ITP

Clinical features of primary and secondary ITP are generally similar, although in secondary ITP clinical manifestations related to the underlying disorder may be prominent. A platelet count below $100 \times 10^9/L$ is required for the diagnosis of ITP because mild thrombocytopenia may occur in normal individuals and uncommonly results in development of more severe thrombocytopenia or other autoimmune disease. The most common symptom of ITP is mucocutaneous bleeding, which may manifest as petechiae, ecchymoses, epistaxis, menorrhagia, oral mucosal, or

gastrointestinal bleeding. In a systematic review, intracranial hemorrhage was reported in 1.4% of adults and 0.4% of children.

Spontaneous bleeding is uncommon at platelet counts $>30 \times 10^9/L$. There is significant variability in bleeding among patients with similar platelet counts, however, and some individuals with counts $<10 \times 10^9/L$ bleed infrequently. The risk of fatal bleeding is greatest in elderly patients with persistent and severe thrombocytopenia ($<20 \times 10^9/L$). Nonhemorrhagic clinical manifestations common among patients with ITP include fatigue and reduced health-related quality of life. Fatigue tracks with platelet count and may improve with platelet-raising therapy in some patients. Mounting epidemiologic evidence suggests that ITP is associated with an increased risk of venous thromboembolism. The mechanism of thrombosis is not well established but may relate to underlying disease pathophysiology and/or treatment effect.

Physical examination should focus on typical bleeding sites. Dependent areas and skin underneath tight clothing should be examined for petechiae and purpura, and oral mucous membranes should be examined for hemorrhagic bullae, which may be associated with an increased risk of severe bleeding at other sites. In a patient with primary ITP, the remainder of the general physical examination is normal. The presence of lymphadenopathy or splenomegaly should prompt investigation for other etiologies of thrombocytopenia. Skeletal, renal, or neurologic abnormalities suggest a familial cause of thrombocytopenia.

Pathophysiology of ITP

Primary ITP is a syndrome that results from several different pathophysiologic mechanisms. Classic experiments performed in the 1950s and 1960s demonstrated a critical role for antiplatelet antibodies in mediating the enhanced clearance of platelets in patients with ITP. These antibodies recognize GPs on the platelet surface, most commonly GPIIb-IIIa and GPIb-IX. Antibody-coated platelets are cleared from the circulation by phagocytes in the reticuloendothelial system, primarily the spleen. Antiplatelet antibodies may recognize the same targets on megakaryocytes, leading to impairment of megakaryocyte proliferation and differentiation and proplatelet production. As noted above, plasma levels of TPO generally are not elevated in patients with ITP due to an expanded megakaryocyte mass and accelerated platelet clearance. Not all patients with ITP have detectable platelet antibodies. Dysregulated T cells may have a direct cytotoxic effect on platelets and impair platelet production by megakaryocytes. Recent interest has focused on decreased levels of regulatory T cells in patients with ITP; successful ITP

treatment has been associated with restoration of regulatory T cell levels.

The pathogenesis of secondary ITP may share similar mechanisms with primary ITP. For example, the thrombocytopenia that occurs in patients with antiphospholipid antibodies may reflect the concurrent presence of antibodies against platelet GPs. Unique pathogenic mechanisms, however, have been identified in some types of secondary ITP. For example, antigen mimicry, in which antibodies directed to a foreign (viral) protein cross-react with specific epitopes on platelet GPIIb-IIIa, has been observed in hepatitis C-associated ITP. A similar pathophysiology may underlie the pathogenesis of ITP in patients with *H. pylori* infection and HIV.

Diagnosis of ITP

The diagnosis of ITP rests on a consistent clinical history, physical examination, and exclusion of other causes of thrombocytopenia. The leukocyte count is characteristically normal. The hemoglobin concentration is typically normal as well, unless thrombocytopenic bleeding has resulted in anemia. Examination of the peripheral blood film should be performed to exclude pseudothrombocytopenia (ethylenediaminetetraacetic acid-dependent platelet agglutinating antibodies), microangiopathic hemolytic anemia (fragmented red cells), or abnormalities suggestive of other disorders. Identification of unexpected abnormalities should prompt an evaluation for other etiologies of thrombocytopenia.

The mean platelet volume may be increased in patients with ITP. However, ITP patients always show a heteroge-

neous platelet population with not more than ~30% enlarged platelets. If the blood smear shows more than 60% large or even giant platelets, hereditary macrothrombocytopenia (see “Hereditary thrombocytopenia” in this chapter) is more likely.

Bone marrow examination is not required routinely and is generally not useful for diagnosing ITP, but should be performed to exclude other causes of thrombocytopenia when atypical features such as unexplained anemia, lymphadenopathy, or splenomegaly are present. Because at least 80% of patients with ITP respond to initial therapy with corticosteroids, intravenous immunoglobulin (IVIg), or Rh-immune globulin (anti-D), failure to respond to these agents should prompt consideration of bone marrow examination and other causes of thrombocytopenia. Bone marrow examination may also be warranted in elderly patients in whom myelodysplasia is suspected. Megakaryocyte number is typically normal or increased in the marrow of patients with ITP. In a blinded study, hematopathologists were not able to reliably distinguish ITP marrows from those of nonthrombocytopenic controls.

With appreciation that secondary causes of ITP may be more common than previously believed and may influence management, additional laboratory studies such as screening for hepatitis C and HIV should be considered. Table 11-2 contains a list of suggested screening studies proposed by the ITP International Working Group. In our practice, we perform a basic evaluation including a history, physical examination, complete blood cell and reticulocyte count, examination of the peripheral blood smear, ABO-Rh blood type, and HIV and

Table 11-2 International Working Group recommendations for the diagnosis of ITP in adults

Basic evaluation	Tests of potential utility	Tests of uncertain benefit
Patient and family history	Glycoprotein-specific antibodies	TPO levels
Physical examination	Antiphospholipid antibodies	Reticulated platelets
CBC and reticulocyte count	Antithyroid antibodies and thyroid function	Platelet-associated IgG
Peripheral blood film	Pregnancy test in women of childbearing potential	Platelet survival study
Bone marrow exam	PCR for parvovirus and CMV	Bleeding time
Blood group (Rh)		Complement levels
Direct antiglobulin test		
<i>H. pylori</i> , HIV, HCV (suggested by majority regardless of geographic region)		
Quantitative immunoglobulins (consider in children with ITP; recommend in children with persistent or chronic ITP)		

Adapted from Provan D et al. *Blood*. 2010;115:168–186.

CBC, complete blood count; CMV, cytomegalovirus; HCV, hepatitis C virus; PCR, polymerase chain reaction.

hepatitis C testing in all patients. Additional tests are requested in selected patients based on the findings of the basic evaluation.

Management of primary ITP in children

Because spontaneous recovery is expected in most children with primary ITP, families of children generally need counseling and supportive care rather than specific drug therapy. Severe hemorrhage occurs in ~1 in 200 children with newly diagnosed ITP, and intracerebral hemorrhage occurs in <1 in 500. For those in whom treatment is considered necessary, a short course of corticosteroids, IVIg, or anti-D (in Rh-positive individuals) generally results in rapid recovery of the platelet count. Adverse effects of therapy in children include behavioral changes from corticosteroids, headache from IVIg, and hemolysis from anti-D, which rarely may be severe. Patients (adults and children) with a positive direct antiglobulin test should not receive anti-D because of an increased risk of severe hemolysis.

Recovery of the platelet count ultimately occurs in >80% of children even without therapy, usually within 3 to 6 months but occasionally over a year or more after presentation. The remaining 20% have chronic ITP (defined as ITP lasting >12 months), yet even in this group, major bleeding is uncommon. Splenectomy is generally reserved for severe persistent or chronic thrombocytopenia with bleeding and results in complete remission in ~75% of children. The risk for overwhelming sepsis after splenectomy is greater in young children, and therefore, splenectomy generally is deferred until at least 5 years of age. Vaccination against *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b should be given before splenectomy in children and adults, and penicillin prophylaxis is recommended until adulthood. Rituximab may also be used as a second-line therapy with initial response rates of 40% to 50%, but a long-term response rate of <25%. Eltrombopag, an oral thrombopoietin receptor agonist (TRA), has been approved by the FDA for use in children >1 year of age with chronic ITP who have failed a prior ITP therapy and require additional treatment to raise the platelet count. Randomized controlled trials in pediatrics have shown a 60% to 75% response rate with a 30% to 40% rate of sustained response. Although it is not approved for use in children, there is also significant experience with romiplostim in pediatric ITP with similar response rates.

Management of primary ITP in adults

In contrast to children, ITP in adults evolves into a chronic disease in approximately 75% of patients. The goal of ITP management in adults is to maintain a hemostatic plate-

let count while minimizing the toxicity of therapy. There are no controlled studies demonstrating the superiority of any specific treatment algorithm and significant variability exists among treatment approaches advocated by different experts. Asymptomatic patients with mild or moderate thrombocytopenia and no bleeding require no specific treatment. Platelet counts $<30 \times 10^9/L$ may be associated with an increased bleeding risk. This platelet count threshold has been suggested by some experts as a cutoff for considering treatment of ITP. However, there is significant variability in bleeding among patients and therapy should be individualized. Treatment decisions should not be dictated by the platelet count alone, but should take into account other factors, including the individual patient's bleeding phenotype; the need for concomitant antithrombotic therapy or other medications that affect hemostasis; the need for an invasive procedure or surgery; lifestyle; comorbidities; and patient values and preferences, including a desire to participate in sports or other activities associated with bleeding risk. Even in asymptomatic or minimally symptomatic patients, an initial short-term treatment course is reasonable to support the diagnosis of ITP and to identify a treatment to which the patient responds in case of worsening symptoms or the need for an invasive procedure.

Although several first-line therapies are available, corticosteroids remain the initial treatment of choice because of their efficacy and low cost. At least 75% of patients initially respond to corticosteroids, although tapering usually precipitates relapse, and ultimately only 20% to 25% of patients are able to maintain a durable platelet response after steroid discontinuation. Standard corticosteroid regimens include prednisone 1 mg/kg/day (typically tapered over ~6 weeks) and high-dose dexamethasone (40 mg daily for 4 days given over 1 to 6 cycles repeated every 2 to 4 weeks). Two controlled trials have compared high-dose dexamethasone alone or in combination with rituximab in newly diagnosed ITP. Combination therapy was associated with superior response rates at 6 and 12 months, but the difference waned with longer-term follow-up, and grade 3 and 4 adverse events were greater in the combination therapy arms. Approximately 25% of patients with ITP may achieve a durable remission after treatment with corticosteroids, usually within the first year after presentation. This observation has led to a recommendation by the International Working Group that splenectomy be deferred until at least 1 year after presentation, if possible.

For patients who do not achieve a response with corticosteroids, therapy may be supplemented with intermittent IVIg or anti-D. Anti-D should only be considered in Rh-positive patients with an intact spleen who have

a negative direct antiglobulin test. Both agents are associated with response rates similar to those of corticosteroids; however, the duration of response is generally only 2 to 4 weeks and thus frequent, intermittent dosing is required if these agents are used for chronic therapy. One uncontrolled study of 28 Rh-positive, nonsplenectomized adults reported that repeated dosing of anti-D for platelet counts $<30 \times 10^9/L$ was an effective maintenance therapy and that 43% of patients treated in this manner ultimately entered a durable remission. Nevertheless, both IVIg and anti-D generally are considered to be bridging agents used to maintain platelet counts in a hemostatic range until more definitive therapy can be initiated.

Second-line therapy is indicated for patients who do not respond to first-line therapy or relapse after it is tapered. Options for second-line therapy include rituximab, TRAs, or splenectomy. Splenectomy has been used to treat ITP for decades, although the availability of alternative treatments, concerns about adverse events, and the realization that some patients with newly diagnosed ITP ultimately may improve over time, has led to decreased utilization in contemporary cohorts compared with older series. Although both the ITP International Working Group and the revised ASH guidelines consider splenectomy an acceptable second-line therapy for ITP, the former group weighs splenectomy equally against other medical options, whereas the ASH guidelines *recommend* splenectomy (grade 1B evidence) for patients who fail corticosteroids while *suggesting* rituximab or TRAs (grade 2C evidence). Splenectomy leads to a high rate of durable remission. In a systematic review, 1,731 (66%) of 2,623 adults with ITP achieved a complete response following splenectomy at a median follow-up of 28 months (range, 1 to 153 months), and this response rate was maintained at 10 years after splenectomy. Splenectomy does not jeopardize subsequent responses to other ITP therapies (other than anti-D) and may reduce long-term costs of ITP management. Disadvantages of splenectomy include a lack of validated predictors of response; surgical risk with a 30-day mortality and complication rate of 0.2% and 9.6%, respectively, for laparoscopic splenectomy and 1.0% and 12.9%, respectively, for open splenectomy; an increased risk of postsplenectomy infection; and an increased risk of vascular thrombosis. The incidence of infection may be reduced by presplenectomy pneumococcal, meningococcal, and *Haemophilus influenzae* b vaccination; repeat pneumococcal vaccination 5 years after initial vaccination; and antibiotic prophylaxis for fever.

Rituximab, an anti-CD20 monoclonal antibody that rapidly depletes CD20⁺ B lymphocytes, may be used in

lieu of splenectomy or in patients who have failed splenectomy. The usual dose is 375 mg/m² weekly for 4 weeks, although an optimal dosing regimen has not been defined and lower doses have shown similar efficacy. In a systematic review of 313 ITP patients, half of whom were not splenectomized, 62.5% achieved a platelet count response (platelet increment of $50 \times 10^9/L$), with a median time to response of 5.5 weeks (range, 2 to 18 weeks) and a median duration of response of 10.5 months (range, 3 to 20 months). In a single-arm study of 60 nonsplenectomized ITP patients, 40% achieved a platelet count $\geq 50 \times 10^9/L$ with at least a doubling from baseline at 1 year, and in 33.3%, this response was sustained for 2 years. An appealing aspect of rituximab therapy is the potential induction of long-term responses in a subset of patients, though long-term remission rates have generally been disappointing. In a long-term follow-up study, only 21% of adults treated with rituximab remained free of relapse at 5 years. In a recently published randomized placebo-controlled trial, there was no benefit of rituximab compared with placebo by 18 months after treatment. Adverse effects of rituximab include infusion reactions (eg, hypotension, chills, and rash), serum sickness, and cardiac arrhythmias. Reactivation of latent JC virus causing progressive multifocal leukoencephalopathy has been reported, but it appears to be extremely uncommon. Reactivation of hepatitis B after rituximab has been described and active hepatitis B infection is a contraindication to treatment. Rituximab also interferes with the response to polysaccharide vaccines. This is of potential concern in patients who may subsequently undergo splenectomy and supports the practice of administering immunizations prior to rituximab.

The TRAs romiplostim and eltrombopag are approved in many countries for patients with ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy. These agents bind and activate the TPO receptor, c-Mpl, leading to increased platelet production. However, they have no structural similarity to endogenous TPO and do not stimulate cross-reactive TPO antibodies. The response rates to these agents range from 59% to 88% and loss of response while on continued therapy is uncommon. These agents are effective before and after splenectomy and usually allow decreases in dosage or discontinuation of concomitant ITP therapy. A disadvantage of these agents is the need for indefinite therapy and the associated costs, although anecdotal reports describe patients in whom these drugs have been discontinued with maintenance of hemostatic platelet counts. Increased bone marrow reticulin develops in approximately 5% of

patients treated with TRAs, but there is no evidence for development of progressive or irreversible bone marrow fibrosis. Eltrombopag carries a boxed warning because of the potential for hepatotoxicity. Patients treated with either agent may develop severe thrombocytopenia following discontinuation of treatment. There may be an increased risk of thrombosis in patients with preexisting risk factors. Thrombotic risk does not appear to be linked to the platelet count.

For patients who do not respond to or are intolerant of second-line therapy, various immunosuppressant medications are available—including azathioprine, cyclosporine, mycophenolate mofetil, cyclophosphamide, vinca alkaloids, dapsone, and danazol. Evidence on use of these agents is limited to uncontrolled case series. Thrombocytopenia in patients with secondary ITP may respond to treatment of the underlying disease. For example, treatment of HIV with antiretroviral therapy induces a platelet response in most patients. Eradication of *H. pylori* has led to resolution of ITP in >50% of cases in certain countries, including Japan, although it has generally not been effective in North America. This may reflect differences in endemic *H. pylori* strains in different geographic regions.

Iron-deficiency anemia is common in ITP, particularly in menstruating women. In addition to platelet-raising therapy, an important element of management is correction of iron deficiency and anemia because a normal red blood cell count improves hemostasis, probably through rheological factors that bring platelets into closer proximity to the endothelium in flowing blood.

Emergency treatment of ITP

Patients with new-onset, severe thrombocytopenia (<20 × 10⁹/L) and bleeding should be hospitalized. Examination of the peripheral blood smear to exclude thrombotic microangiopathy and a careful medication history to exclude drug-induced thrombocytopenia should be undertaken. Once a presumptive diagnosis of ITP has been reached, management of bleeding may require platelet transfusions in combination with high doses of parenteral corticosteroids (methylprednisolone 1 g intravenously daily for 2 to 3 days) supplemented with IVIg (1 g/kg for 1 to 2 days). Increases in the platelet count may become apparent within 3 to 5 days, although complete responses may require 1 to 2 weeks. All-trans-retinoic acid (ATRA), vinca alkaloid, or emergency splenectomy may be required for patients with refractory thrombocytopenia and persistent bleeding. In case of life-threatening bleeding, massive platelet transfusion can control hemorrhage, but typically multiple platelet units are required.

KEY POINTS

- ITP may occur as a primary disorder or secondary to a predisposing illness.
- The diagnosis of primary ITP is made by excluding other causes of thrombocytopenia.
- ITP in children is usually self-limited; conversely, ITP in adults develops into a chronic disease in ~75% of patients.
- The pathogenesis of ITP involves accelerated platelet destruction and decreased platelet production.
- Corticosteroids, supplemented as needed with IVIg or anti-D, are first-line therapy for ITP.
- Second-line therapy (splenectomy, rituximab, TRAs) is indicated in patients who do not respond to first-line therapy or relapse after it is tapered.
- Emergency treatment of severe ITP includes a combination of steroids and IVIg and, in case of life-threatening bleeding, massive platelet transfusion.

Drug-induced immune thrombocytopenia (DITP)

More than 300 drugs have been implicated in drug-induced immune thrombocytopenia (DITP), including quinine and quinidine (present in tonic water, bitter lemon, and certain medications), nonsteroidal anti-inflammatory agents, trimethoprim-sulfamethoxazole, vancomycin, beta-lactam antibiotics, levofloxacin, rifampin, anticonvulsants, sedatives, and the platelet GPIIb-IIIa inhibitors tirofiban, eptifibatide, and abciximab. A systematic review of individual patient data found that the most commonly reported drugs with a definite or probable causal relation to thrombocytopenia were quinidine, quinine, rifampin, and trimethoprim-sulfamethoxazole. A database of implicated drugs is available online and periodically updated (Platelets on the Web; available at <http://www.ouhsc.edu/platelets>). Heparin-induced thrombocytopenia (HIT) is discussed separately because of its unique clinical manifestations and pathophysiology.

Mechanisms of DITP

DITP characteristically occurs approximately 1 to 2 weeks after initial drug exposure, a timeframe consistent with production of drug-dependent or drug metabolite-dependent IgG antibodies. An exception is thrombocytopenia induced by the GPIIb-IIIa antagonists, eptifibatide, tirofiban, and abciximab, which may present within hours of exposure due to naturally occurring antibodies. Several mechanisms specific for individual drugs underlie the development of DITP. Quinine-induced thrombocytopenia was first described more than a century ago and serves as

a prototype. In this disorder, the binding of antibodies to platelet GPs is greatly enhanced in the presence of the sensitizing drug. This may result from binding of the drug to specific GPs, such as GPIIb-IIIa or GPIb-IX. Affinity maturation of B cells producing low-affinity antibodies reacting with the neoepitope induced by the complex of the drug and the platelet GP may result in the generation of antibodies that can destroy platelets in the presence of the drug. Another potential mechanism is modification of the hypervariable region of the antibody when a small molecule drug binds to the antigen recognition site, thereby modifying the specificity of the antibody.

A much rarer mechanism of DITP involves the induction of autoantibodies by drugs such as gold and interferon- α or - β , leading to development of a syndrome that resembles ITP. An often-overlooked cause of DITP is that which follows vaccinations, including diphtheria-pertussis-tetanus and measles-mumps-rubella, which reflects the development of true autoantibodies similar to those described in ITP.

Tirofiban and eptifibatide (“fibans”) are small molecule mimetics of the RGD region of fibrinogen that inhibit fibrinogen binding to activated GPIIb-IIIa and block platelet aggregation. Thrombocytopenia may occur because of pre-existing antibodies that recognize conformation-dependent neoepitopes (mimetic-induced binding sites induced in GPIIb-IIIa following drug binding (rapid onset), as well as by induction of new antibodies toward the neoepitope induced by fiban binding to the GPIIb-IIIa complex (delayed onset). Abciximab, a chimeric (mouse–human) Fab fragment to GPIIb-IIIa, causes acute profound thrombocytopenia in 0.5% to 1.0% of patients on their first exposure because of preexisting antibodies that recognize the murine portion of abciximab. About 50% of cases of “fiban-induced thrombocytopenia” are due to pseudothrombocytopenia. The combination of fiban binding and calcium depletion by the anticoagulant enhances binding of natural IgM antibodies in the test tube, resulting in platelet clumping. Fiban-induced pseudothrombocytopenia frequently also manifests in citrated blood. As fibans are given in patients with high-risk coronary interventions, recognition of pseudothrombocytopenia is of major importance. Inappropriate cessation of antiplatelet therapy, and potentially even platelet transfusion or other prohemostatic measures, may subject the patient to an increased risk of thrombosis including in-stent thrombosis.

Diagnosis of DITP

Clinical criteria have been proposed that may be used to judge the likelihood of a given drug causing DITP. These include a temporal association between drug exposure and thrombocytopenia, the exclusion of other causes of

thrombocytopenia, and recurrence of thrombocytopenia upon drug rechallenge. In practice, particularly in hospitalized patients, a multitude of potential culprit drugs and concurrent illnesses (such as infections) may make the diagnosis of DITP difficult. An important diagnostic clue is the timing of initiation of the drug. Typically, the causal drug has been started 1 to 2 weeks before the onset of thrombocytopenia. In hospitalized patients, antibiotics are the most frequent cause of DITP. Specialized laboratory assays for antibodies that bind to platelets in the presence of a drug or drug metabolite have been developed. However, such assays are only available for a limited number of drugs and drug metabolites, are not standardized, and are only performed at a small number of reference laboratories around the world. They may provide useful confirmation of DITP, but because there is a several-day turnaround time for these “send-out” tests, clinicians are forced to make critical initial decisions about whether to suspend suspicious medications without the benefit of laboratory results.

DITP is characteristically severe, with a median nadir platelet count of approximately $20 \times 10^9/L$ and a high risk of hemorrhage. A review of 247 case reports of DITP found an incidence of major and fatal bleeding of 9% and 0.8%, respectively.

Treatment for DITP involves discontinuation of the offending drug. A practical approach in hospitalized patients on multiple medications is to stop all drugs started within the last 2 weeks (excluding electrolytes and nutrients), when feasible, and to switch antibiotics. The platelet count starts to recover after 4 to 5 half-lives of the culprit drug or drug metabolite, which can last several days. Patients with severe thrombocytopenia and bleeding, as well as those judged to be at particularly high risk of bleeding, may be treated with IVIg, corticosteroids, or plasma exchange, though there is only limited evidence to support these interventions. Platelet transfusion is generally ineffective. Patients should be instructed to avoid the culprit drug in the future, and it should be added to their allergy list.

KEY POINTS

- Many drugs have been implicated as causes of DITP.
- Quinidine, quinine, and antibiotics such as trimethoprim-sulfamethaxazole and vancomycin are common culprits.
- Thrombocytopenia caused by tirofiban, eptifibatide, and abciximab may occur soon after exposure in patients not previously exposed to these drugs.
- DITP can be confirmed in some cases by demonstration of drug- (or drug metabolite) dependent, platelet-reactive antibodies *in vitro*.

Heparin-induced thrombocytopenia

HIT is an idiosyncratic drug reaction caused by antibodies against multimolecular complexes of PF4 and heparin. Binding of HIT antibodies to Fc receptors on monocytes and platelets causes cellular activation; HIT antibodies also activate endothelial cells by binding endothelial cell-associated PF4. The net result is elevated levels of circulating microparticles and an intensely prothrombotic state. HIT occurs most commonly in patients receiving unfractionated heparin (UFH). The incidence of HIT in the in-hospital patient population is about 1 in 5,000, but it varies widely among patient groups, with reported incidences of 0.2% to 5.0% in patients receiving UFH. The risk of HIT associated with low-molecular-weight heparin (LMWH) is 5- to 10-fold lower. Use of LMWH instead of UFH is the most efficient measure to prevent HIT in any patient group (but LMWH must not be used when HIT has developed). Thrombosis develops in 40% to 50% of patients with HIT. Despite the occurrence of thrombocytopenia, bleeding is rare. Although the diagnosis of HIT in the acute setting is clinical, confirmation depends on correlative laboratory testing. Transient thrombocytopenia following the administration of heparin (previously called type I HIT, or nonimmune HIT) is an innocuous syndrome caused by binding of heparin to platelet GPIIb-IIIa, thereby inducing a signal, which lowers the threshold for platelet activation by other agonists.

Clinical features

HIT is a clinicopathological syndrome that requires both the presence of platelet-activating antibodies, usually directed toward PF4/heparin complexes, and clinical symptoms that include a decrease in the platelet count by >50% and/or new thromboembolic complications. As a general rule, the clinical symptoms manifest between day 5 and 14 after initiation of heparin. An exception is rapid-onset HIT, in which patients with recent heparin exposure (usually within the last 30 days) and preexisting HIT antibodies may manifest clinical HIT within hours of heparin reexposure. A second exception is delayed-onset HIT, which typically presents 2 to 3 weeks after prior heparin exposure. In delayed-onset HIT, the antibodies have gained autoreactivity (ie, they recognize PF4 bound to endogenous glycosaminoglycans on platelets and therefore activate platelets even in the absence of heparin). A platelet count decrease or a new thrombosis without corresponding antibodies is not HIT. Similarly, a positive assay for platelet-activating antibodies or a positive PF4-heparin enzyme-linked immunosorbent assay (ELISA) without corresponding clinical symptoms is not HIT.

HIT is uncommon in children and is more common in females (odds ratio 2.37). The incidence of HIT is approximately 3-fold greater in surgical than in medical patients. While patients receiving thromboprophylaxis with UFH after major orthopedic surgery had the highest incidence of HIT (5%) in the 1990s, today HIT is rare in this patient group. Whether this is related to the widespread use of LMWH or to other changes in surgical practice is unknown. Today, patients with cardiac assist devices and those undergoing cardiac surgery have the highest incidence of HIT (1% to 3%). Absolute thrombocytopenia (platelet count $<150 \times 10^9/L$) is not required for a diagnosis of HIT; rather, a substantial (>50%) decrease in the platelet count from the highest platelet count after initiation of heparin is required. This is particularly relevant to the postoperative setting, in which platelet count values typically rise to 20% to 30% above the preoperative baseline at day 8 to 10 after major surgery. Rarely, HIT can manifest as an autoimmune disorder without any exposure to heparin—so-called spontaneous or autoimmune HIT. PF4 binds to polyanions other than heparin, such as lipopolysaccharide on bacteria or RNA/DNA (released during major surgery), and undergoes the same changes in its conformation as when binding to heparin. These endogenous PF4-polyanion complexes are likely the trigger for autoimmune HIT. The resulting antibodies are typically of very high titer and, in contrast to typical HIT antibodies, can persist for months. A typical characteristic of autoimmune HIT antibodies is that they activate platelets even in the absence of heparin. This feature is used to confirm autoimmune HIT.

Several clinical scoring systems have been developed to assist with determining the pretest probability of HIT. The most commonly used is the 4Ts system (thrombocytopenia, timing, thrombosis, and other; see Table 11-3). This system has been shown to have a high negative predictive value (ie, a low score is useful in ruling out HIT), but its effectiveness is limited by modest interobserver agreement and a relatively low positive predictive value. Recent studies have demonstrated that this system may be of less utility in intensive care patients, a setting in which HIT is often suspected due to the high prevalence of thrombocytopenia, but is relatively uncommon with an incidence of ~0.5%. Another system, the HIT Expert Probability score, has also been developed, although the clinical experience with this system is not as extensive. The impact of either scoring system on patient outcomes has not been determined. While a low 4T score (<4 points) makes HIT unlikely, HIT may nevertheless be the underlying cause in 2% to 3% of patients. Typically, in these patients relevant information is not available (eg, due to transfer from another hospital).

Table 11-3 4Ts scoring system for HIT

4Ts	2 points	1 point	0 point
Thrombocytopenia	Platelet count decrease of >50% and platelet nadir $\geq 20 \times 10^9/L$	Platelet count decrease of 30%–50% or platelet nadir 10 to $19 \times 10^9/L$	Platelet count fall of <30% or platelet nadir $< 10 \times 10^9/L$
Timing of platelet count fall	Clear onset of thrombocytopenia 5–10 days after heparin administration; or platelet decrease within 1 day, with prior heparin exposure within 30 days	Consistent with day 5–10 decrease but not clear (eg, missing platelet counts) or onset after day 10; or decrease within 1 day, with prior heparin exposure 30–100 days ago	Platelet count decrease <4 days without recent exposure
Thrombosis or other sequelae	New thrombosis (confirmed); skin necrosis (lesions at heparin injection site); acute systemic reaction after intravenous unfractionated heparin bolus	Progressive or recurrent thrombosis; nonnecrotizing skin lesions; suspected thrombosis (not proven)	None
Other causes for thrombocytopenia	None apparent	Possible	Definite

Adapted from Lo G et al. *J Thromb Haemost*. 2006;4:759–765.

However, a combination of a low score and a negative PF4-heparin ELISA essentially rules out HIT.

Thrombosis is present in ~50% of newly diagnosed cases of HIT, and it develops in ~40% of patients with asymptomatic thrombocytopenia resulting from HIT within the first 10 days following heparin discontinuation if appropriate treatment is not administered. Venous thrombosis occurs twice as frequently as arterial thrombosis, although limb artery thrombosis, myocardial infarction, and microvascular thrombosis have been described. HIT-associated thrombosis occurs with increased frequency at sites of vessel injury (eg, central venous catheter-associated deep vein thrombosis). For this reason, vascular interventional procedures (other than arterial thrombectomy) and placement of intravascular devices such as vena caval filters should generally be avoided. Adrenal infarction secondary to adrenal vein thrombosis, skin necrosis at heparin injection sites, and anaphylactoid reactions after an intravenous heparin bolus also may occur as a result of PF4/heparin antibodies. Thrombosis in unusual sites, such as cerebral sinuses, vascular grafts, fistulas, and visceral vessels may also develop. Phlegmasia due to occlusion of the lower-extremity venous system resulting in arterial insufficiency is a typical complication when vitamin K antagonists are started too early in HIT (ie, prior to platelet count recovery). The resulting protein C deficiency triggers microvascular thrombosis distal to large vessel thrombosis, which may have occurred as an initial manifestation of HIT. Very severe HIT can be associated with disseminated intravascular coagulation (DIC). Patients with DIC often present with platelet counts below $20 \times 10^9/L$, whereas otherwise a platelet count nadir of $40 \times 10^9/L$ to $80 \times 10^9/L$ is the more typical range for HIT.

HIT testing

Two types of tests are available for detection of HIT antibodies: PF4/heparin immunoassays (eg, PF4/heparin ELISA) and functional assays demonstrating the ability of HIT antibodies to activate washed platelets, such as the serotonin release assay, generally considered the gold standard for diagnosis, or heparin-induced platelet activation (HIPA) test.

The sensitivity of most PF4/heparin immunoassays approaches 100%, and thus a negative test is useful in excluding HIT. Difficulties concerning use of the PF4/heparin ELISA include long turnaround time in institutions in which it is not performed daily and a high false-positive rate, particularly in the postcardiac surgery setting. Specificity may be increased by considering the level of positivity. High ELISA reactivity correlates closely with the presence of platelet-activating HIT IgG, whereas positive platelet activation studies are uncommon in patients with weakly positive ELISA optical density values (0.4 to 0.9). The use of an ELISA that detects only anti-PF4/heparin IgG, as opposed to the polyspecific ELISA that detects IgG, IgA, and IgM antibodies, also increases specificity; as may the addition of a confirmatory step performed in the presence of high heparin concentrations. Recently automated tests for anti-PF4/heparin antibodies have been introduced. They are highly standardized and allow a rapid turnaround time, but some may produce false negative results in about 2% to 3% of patients.

Functional assays have improved specificity compared with immunoassays. These assays are technically difficult, however, requiring washed donor platelets; and for the serotonin release assay, radioisotope. Because of these considerations, the performance of functional assays is limited

primarily to specialized reference laboratories, and their results generally are not available at the time the diagnosis of HIT is considered. They are, however, important for confirming the diagnosis and for long-term management because patients without HIT may be harmed by being incorrectly labeled as having a history of HIT, with consequent avoidance of heparin and unnecessary use of alternative anticoagulants.

Treatment of HIT

Although previously underdiagnosed, increased appreciation of HIT and the frequent use of highly sensitive tests has led to overdiagnosis in the current era, with the attendant costs and increased bleeding risks associated with inappropriate anticoagulation therapy. Current guidelines of the American College of Chest Physicians suggest that routine monitoring of the platelet count in patients on heparin therapy should be performed every 2 to 3 days for patients with a risk of HIT of >1% and that routine monitoring is unnecessary for those in whom the risk of HIT is <1% (Table 11-4). Because typical-onset HIT begins no earlier than day 5 of heparin treatment and reactive thrombocytosis after surgery needs to be considered in order to detect a >50% relative fall in the platelet count, monitoring the platelet count on days 5, 7, and 9 after surgery in high-risk patients is generally sufficient. If the platelet count fall begins on or before day 9, it can be detected by these monitoring time points. When a new thrombosis occurs after day 9, comparison of the platelet

count at the time of thrombosis with the one obtained on day 9 facilitates recognition of HIT, while comparison with the preheparin baseline platelet count could underestimate the magnitude of the platelet count fall. However, platelet count monitoring rarely helps to prevent initial thrombosis in HIT because the time between the fall in platelet count and onset of thrombosis can be very short, or both may occur concomitantly.

The cornerstone of HIT therapy is immediate discontinuation of heparin when the disease is suspected, usually before laboratory diagnosis. Some experts recommend 4-limb ultrasound in patients with HIT because silent DVT is common and may influence the duration of anticoagulation. Anticoagulation using a nonheparin anticoagulant at a therapeutic dose should be initiated, even in patients with no thrombosis, because of the massive thrombin generation in HIT and continued high risk of thrombosis after heparin discontinuation. Alternative anticoagulation in patients without thrombosis should be continued until the platelet count has recovered. Some advocate a longer duration of anticoagulation (eg, 30 days), although no controlled data demonstrating the benefit of this approach are available. Patients with HIT and thrombosis should receive at least 3 months of therapeutic dose anticoagulation. LMWH must not be used because of cross-reactivity with most heparin-dependent antibodies. Warfarin must not be given in acute HIT. It may be started once the platelet count has reached a stable plateau, indicating that the acute prothrombotic process is under control, but only with appropriate overlap with an alternative parenteral anticoagulant. Warfarin leads to hypercoagulability because of the inhibition of protein C γ -carboxylation, which increases the risk for microvascular thrombosis. In patients who develop HIT while taking warfarin, warfarin should be discontinued, an alternative nonheparin anticoagulant should be initiated, and vitamin K should be administered with the goal of repleting protein C and preventing microvascular thrombosis.

Currently available nonheparin anticoagulants in the United States include the parenteral direct thrombin inhibitors, argatroban and bivalirudin, as well as fondaparinux and the direct oral anticoagulants. Argatroban is hepatically cleared and approved for treatment of HIT with or without thrombosis, as well as percutaneous coronary intervention in patients with HIT or at risk for HIT. The use of argatroban in HIT is associated with a hazard ratio of 0.3 for the development of new thrombosis. Argatroban is monitored using the activated partial thromboplastin time (aPTT), but also raises the prothrombin time/international normalized ratio. Thus, transitioning patients from argatroban to warfarin should be performed

Table 11-4 Incidence of HIT according to patient population and type of heparin exposure

Patient population (minimum 4 days' exposure)	Incidence of HIT (%)
Postoperative patients	
Heparin, prophylactic dose	1–5
Heparin, therapeutic dose	1–5
Heparin, flushes	0.1–1.0
LMWH, prophylactic or therapeutic dose	0.1–1.0
Cardiac surgery patients	1–3
Medical	
Patients with cancer	1.0
Heparin, prophylactic or therapeutic dose	0.1–1.0
LMWH, prophylactic or therapeutic dose	0.6
Intensive care patients	0.4
Heparin, flushes	<0.1
Obstetric patients	<0.1

Adapted from Linkins LA et al. *Chest*. 2012;141(2 suppl):e495S–e530S.

by following the guidelines suggested by the manufacturer. Bivalirudin is approved for percutaneous coronary interventions in patients with HIT or a history of HIT and has the advantage of a short half-life of only 25 minutes. A limitation of both argatroban and bivalirudin is that they are subject to aPTT confounding, a phenomenon in which patients with clotting factor deficiency (due to liver impairment, warfarin treatment, or consumptive coagulopathy) have resultant prolongation of the aPTT, leading to underdosing of anticoagulation. Use of the dilute thrombin time assay rather than the aPTT provides more reliable results. Other anticoagulants, such as danaparoid and lepirudin, are no longer available in the United States.

A number of reports have described the favorable use of the synthetic pentasaccharide fondaparinux in patients with HIT, although this agent has not been studied in a controlled manner. The direct oral FXa inhibitors (rivaroxaban, apixaban, and edoxaban) or the direct oral thrombin inhibitor dabigatran may be an option in HIT, but apart from case series, no systematic data are available. Because the plasma levels of these drugs change considerably between peak and trough, there is a risk that the highly prothrombotic state of acute HIT could lead to breakthrough thrombosis at drug trough levels. The oral direct thrombin and FXa inhibitors may be used once the acute phase of HIT is resolved (as signified by platelet count recovery) or in patients with a history of HIT. In patients with autoimmune HIT, alternative anticoagulants must be maintained in therapeutic dose until the platelet count has reached normal levels, which may last several months. Recently, several patients with autoimmune HIT have been shown to respond with a rapid and persistent increase of the platelet count upon treatment with high-dose IVIg (1g/kg/day for 2 consecutive days). In part, high-dose IVIg blocks activation of the platelet Fc receptor by HIT antibodies, but it may also have an immune-modulatory effect. IVIg does not have anticoagulant activity and so it must be given with a nonheparin anticoagulant.

HIT antibodies are transient and typically vanish within 3 months after discontinuation of heparin. Once antibodies disappear (ie, HIT laboratory testing becomes negative), it is safe to re-expose patients to heparin during a cardiovascular procedure or surgery. Heparin must be limited to the intraoperative setting and scrupulously avoided before and after surgery. If cardiovascular surgery is required in a patient with HIT and the procedure cannot be delayed until HIT antibodies disappear, options for intraoperative anticoagulation include use of a nonheparin parenteral anticoagulant (eg, bivalirudin), plasma exchange (using plasma as the replacement fluid) to reduce HIT an-

tibody titers and allow heparin use, or use of intraoperative heparin in combination with a prostacyclin analogue.

KEY POINTS

- HIT occurs in 0.2% to 5% of adults exposed to UFH, approximately 40% to 50% of whom develop thrombosis.
- HIT antibodies are directed against large multimolecular complexes of PF4 and heparin (or other polyanions).
- Systematic scoring systems facilitate estimation of the pretest probability of HIT. A low 4T score (<4 points) makes HIT very unlikely and, together with a negative PF4-heparin ELISA, rules out HIT.
- Functional assays including the serotonin release assay and the HIPA test are useful for confirming the diagnosis of HIT.
- When HIT is suspected, heparin must be discontinued and a nonheparin anticoagulant initiated in therapeutic dose (unless there is a substantial risk for bleeding).
- Warfarin must not be started in acute HIT, but may be initiated for long-term anticoagulation once the platelet count has normalized at a stable plateau for 2 consecutive days.

Other causes of thrombocytopenia

Thrombotic microangiopathies

CLINICAL CASE

A 17-year-old female is referred for evaluation of renal insufficiency and anemia. She and her siblings were placed in foster care while they were very young and she has no information on the health of her parents or older relatives. Her renal function was first noted to be abnormal 1 year ago and over the last 2 months she has developed profound fatigue. Her 22-year-old sister is married and in good health. Her 15-year-old brother also has been noted to have mildly abnormal renal function as well as significant anemia. On examination, she appears fatigued and pale. There is no organomegaly. The complete blood count reveals a hemoglobin of 8.5 g/dL, a WBC of $9.1 \times 10^9/L$, and a platelet count of $77 \times 10^9/L$. The lactic dehydrogenase (LDH) is elevated at 632 IU/L. The peripheral blood film reveals 1 to 2 schistocytes per high-power field. Subsequent evaluation including sequencing of complement regulatory genes reveals a mutation in factor H.

Clinical features

The thrombotic microangiopathies discussed in this chapter include TTP and the typical and atypical hemolytic

uremic syndromes (HUS and aHUS, respectively). Each of these disorders is characterized by microangiopathic hemolytic anemia (MAHA) and thrombocytopenia, with a variable component of neurologic or renal dysfunction and fever. This pentad of symptoms was once common at the time of presentation, but increased awareness of these disorders has led to earlier diagnosis. Currently, the presence of schistocytic anemia and thrombocytopenia is sufficient for the diagnosis of thrombotic microangiopathy (TMA).

TTP occurs in both a rare inherited form called Upshaw-Schulman syndrome due to biallelic mutations in the VWF-cleaving protease, ADAMTS13 (a disintegrin and metalloprotease with thrombospondin-1-like repeats), as well as a more common acquired form in which ADAMTS13 deficiency is caused by autoantibodies. Patients with TTP generally present acutely or subacutely with fatigue and malaise, with variable neurologic symptoms that may range from mild personality changes to obtundation. Renal insufficiency may or may not be present. aHUS presents in a similar manner, but it may demonstrate a more chronic presentation with progressive renal insufficiency, low-grade MAHA, and thrombocytopenia. Neurologic defects are less common in aHUS than in TTP. Typical HUS follows infection with enteropathogenic *Escherichia coli*, may occur in epidemics, and often is preceded by bloody diarrhea and abdominal pain. Not all patients with typical HUS have diarrhea, however, whereas up to 30% of aHUS patients may provide such a history; thus, the presence or absence of diarrhea does not always distinguish these disorders. Renal insufficiency is usually the most prominent component of typical HUS.

Distinguishing between different TMAs may be difficult because of extensive overlap in symptoms. A recently validated scoring system, the PLASMIC score, was developed to identify patients with TTP. Recent scientific advances have led to new information concerning the pathogenesis of TMAs and development of diagnostic tests. For example, TTP is associated with deficiency of ADAMTS13, while mutations in complement regulatory proteins can be identified in 50% to 70% of cases of aHUS. These findings have facilitated the development of pathogenesis-based classification schemes for TMAs. An example of one scheme developed by the British Committee for Standards in Haematology and the British Transplantation Society is depicted in Table 11-5.

Pathogenesis

TMAs cause microvascular thrombi in critical organs, leading to ischemia and organ damage. These thrombi induce shearing of red blood cells, leading to the characteristic schistocytic anemia. Endothelial cell activation or

Table 11-5 Classification scheme for thrombotic microangiopathies

Disorders in which etiology is established
ADAMTS13 abnormalities
ADAMTS13 deficiency secondary to mutations
Antibodies against ADAMTS13
Disorders of complement regulation
Genetic disorders of complement regulation
Acquired disorders of complement regulation (eg, factor H antibody)
Infection induced
Shiga toxin—and verotoxin (Shiga-like toxin)—producing bacteria
<i>Streptococcus pneumoniae</i>
Defective cobalamin metabolism
Quinine induced
Disorders in which etiology is not well understood
HIV
Malignancy
Drugs
Pregnancy
Systemic lupus erythematosus and antiphospholipid syndrome

Adapted from Taylor CM et al. *Br J Haematol*. 2009;148:37–47.

damage also promotes TMA, leading to the elaboration of unusually large VWF multimers that enhance platelet aggregation and microvascular occlusion.

TTP results from an inherited or acquired deficiency of ADAMTS13, leading to elevated levels of unusually large VWF multimers that induce platelet aggregation in the microvasculature. ADAMTS13 regulates VWF activity by cleaving high-molecular-weight multimers; failure to do so may result in the microvascular thrombosis and ischemia characteristic of TTP (Figure 11-2). The observation that some patients with ADAMTS13 deficiency do not have clinical manifestations of TTP suggests that factors other than ADAMTS13 deficiency, such as endothelial damage or activation, are also needed to trigger TTP. Other TTP-like syndromes can be caused by drugs—including quinine, ticlopidine, clopidogrel, cyclosporine, tacrolimus, mitomycin C, and gemcitabine—or may occur in the setting of bone marrow transplantation, systemic lupus erythematosus, disseminated malignancy, and HIV infection. The pathogenesis of these syndromes is diverse; whereas some are associated with antibodies to ADAMTS13, others are not and may result from direct endothelial cell toxicity.

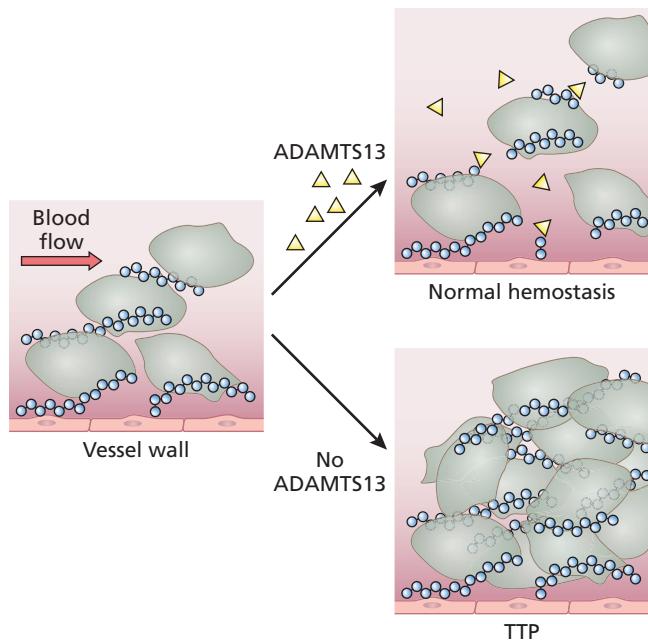


Figure 11-2 Pathogenesis of TTP caused by ADAMTS13 deficiency. Multimeric VWF adheres to endothelial cells or to connective tissue exposed in the vessel wall. Platelets adhere to VWF through platelet membrane GPIb-IX. In flowing blood, VWF in the platelet-rich thrombus is stretched and cleaved by ADAMTS13, limiting thrombus growth. If ADAMTS13 is absent, VWF-dependent platelet accumulation continues, eventually causing microvascular thrombosis and TTP. Redrawn from Sadler JE. *Blood*. 2008;112:11–18.

Typical HUS results from infection by enteropathogenic *E. coli*, most commonly serotype O157:H7. The capacity of organisms to cause HUS reflects their production of two 70-kDa bacterial exotoxins called verotoxins. Verotoxin-1 is homologous to a *Shigella* toxin and therefore generally is referred to as Shiga-like toxin 1 (SLT-1 or Stx1). Most strains of pathogenic *E. coli* produce a second toxin, Stx2, which is associated with a higher risk of developing HUS. The intact 70-kDa Stx holotoxin consists of a 32-kDa A subunit and five 7.7-kDa B receptor-binding subunits that bind globotriaosylceramide (Gb3; CD77) receptors expressed on capillary endothelium. Following binding to Gb3, the toxin is internalized. The A subunit is proteolyzed to a 27-kDa A1 subunit that binds the 60s ribosomal subunit, inhibiting protein synthesis and inducing endothelial cell apoptosis. Recent studies have demonstrated that signal transduction initiated through cross-linked Stx B subunit/Gb3 complexes induce the release of VWF from endothelial cells. Finally, Stx acts in concert with lipopolysaccharide to trigger a procoagulant state that involves platelet activation, tissue factor induction, and the release of unusually large VWF multimers.

The pathogenesis of aHUS reflects increased activation of the alternative complement pathway (AP) because of mutations or autoantibodies resulting in loss or functional impairment of complement regulatory proteins; or, less frequently, activating mutations in complement proteins themselves. Most hereditary forms of aHUS are transmitted in an autosomal dominant manner, although penetrance is only 50%. Under normal conditions, the AP is constitutively activated because of ongoing C3 hydrolysis (Figure 11-3), and thus tight regulation of the AP by complement inhibitory proteins is required to prevent complement-mediated injury. AP activation leads to the generation of the C5b-C9 lytic complex on cell surfaces, and in the case of aHUS, endothelial cell damage is the primary consequence, resulting in characteristic microvascular thrombotic lesions. Complement activation is regulated primarily by the plasma protein, factor H, and the membrane-associated membrane cofactor protein (MCP; CD46); each of which binds membrane-bound C3b and promotes its inactivation by factor I. Several mutations in complement regulatory proteins underlie the development of aHUS. Most common are mutations in factor H, which impair the interactions of factor H with membrane-bound C3b, and account for 30% of cases; an additional 5% to 10% of cases of aHUS result from acquired antibodies to factor H. Mutations in CD46, usually impairing membrane expression, are observed in 15% of patients with aHUS. Factor I mutations occur in 12% of aHUS patients. Activating mutations in factor B or C3 occur in 5% to 10% of patients with aHUS. Mutations in thrombomodulin, another complement regulatory protein, have been described.

Diagnosis

The diagnosis of TMA requires clinical awareness and prompt recognition of symptoms. TTP is more common in females, with a peak incidence in the fourth decade; other risk factors include obesity and African ancestry. The diagnosis of TTP should be suspected in patients with MAHA and thrombocytopenia without another apparent etiology, such as malignant hypertension, vasculitis, scleroderma renal crisis, tumor emboli, or DIC. Fever and neurologic symptoms may be present but are less common than they once were due to earlier diagnosis; evidence of renal involvement even in the absence of renal insufficiency sometimes can be obtained through examination of the urinary sediment. Schistocytes are almost invariably present and accompanied by elevation of the LDH, which may be striking; levels of unconjugated bilirubin also may be increased. Nucleated red blood cells are frequently present. The PT, aPTT, and fibrinogen levels

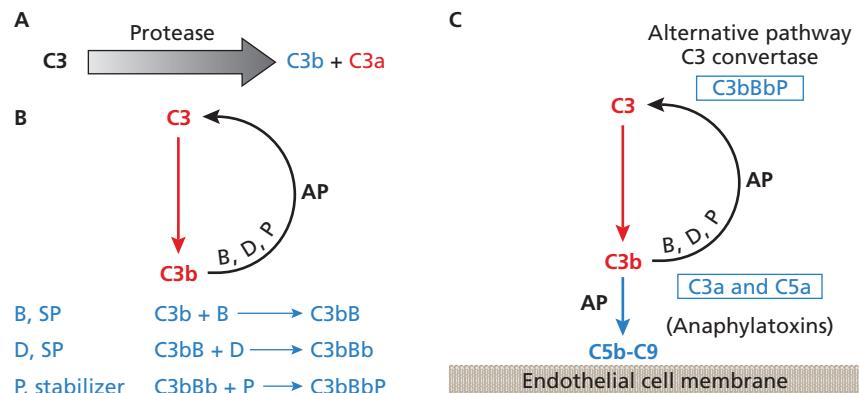


Figure 11-3 The alternative pathway of complement activation. (A) The AP of the complement system originally consisted of a serine protease that cleaved C3 to the opsonin C3b and the proinflammatory anaphylatoxin C3a. (B) An amplification loop was next evolved to more efficiently deposit C3b on a target and liberate C3a into the surrounding milieu. B indicates factor B; D indicates factor D, a serine protease; P, properdin, a stabilizer of the enzyme. (C) Development of a C5 convertase. The same enzyme that cleaves C3 (AP C3 convertase) can cleave C5 to C5a and C5b with the addition of a second C3b to the enzyme complex (AP C5 convertase). Redrawn from Liszewski MK, Atkinson JP. *Hematology Am Soc Hematol Educ Program*. 2011;2011:9–14. AP, alternative complement pathway.

are typically normal, and the D-dimer is normal or only mildly increased. The direct antiglobulin test is negative. Consideration of secondary causes of TTP should include a detailed drug history, HIV testing, and a focused search for autoimmune disease and malignancy. TTP may present during pregnancy, particularly in the second and third trimesters. ADAMTS13 activity assays may be useful in confirming the diagnosis of TTP when severe deficiency (<10%) is present in the appropriate clinical setting. ADAMTS13 testing may also provide prognostic information, with lower levels of ADAMTS13 and higher levels of anti-ADAMTS13 antibodies associated with higher relapse rates. Some patients with a TMA other than TTP and detectable or even normal ADAMTS13 levels, however, also respond to plasma exchange; and thus, this therapy should not be withheld from such individuals. Moreover, recovery of ADAMTS13 levels during initial plasma exchange may lag behind clinical response and is not useful in determining the duration of plasma exchange.

Patients with aHUS may present acutely, mimicking TTP, or in some cases more insidiously with renal insufficiency as the primary symptom. Thrombocytopenia may be less severe in aHUS than TTP. A family history of similar disease may be apparent, although the low penetrance of complement inhibitor mutations make such a history difficult to dissect. Exacerbations of disease may follow infections and may be accompanied by fatigue and malaise. aHUS may present in association with pregnancy, most commonly at 3 to 4 weeks postpartum. Comple-

ment levels in patients with aHUS may be decreased, but normal levels do not exclude aHUS. Sequencing of complement inhibitor proteins is useful for confirming a clinical impression of aHUS, but 30% to 40% of patients who respond to complement inhibition do not have an identifiable mutation and genetic variants of unknown significance are common.

Typical HUS is more common in the pediatric population than in adults, and it is the most common cause of acute renal failure in children. The disease begins with abdominal pain and watery diarrhea 2 to 12 days after toxin exposure. Bloody diarrhea generally ensues on the second day, though up to one-third of patients do not report blood in the stool. Fever is typically absent or mild. The presentation may be difficult to differentiate from inflammatory bowel disease, appendicitis, ischemic colitis, or intussusception. Definitive diagnosis is made by culture of *E. coli* on sorbitol-MacConkey agar. The presence of Shiga toxin or its structural genes may be detected by enzyme immunoassay or polymerase chain reaction of the stool. Serologic studies demonstrating an increase in convalescent antibody titer to Shiga toxin or *E. coli* lipopolysaccharide may be useful in confirming the diagnosis.

Management

Plasma exchange is the standard of care for treatment of TMAs, particularly TTP. Untreated, TTP is associated with a mortality of approximately 85%, although 90% of patients with TTP treated with plasma exchange

survive. The superiority of plasma exchange over infusion was demonstrated in a randomized Canadian trial of 103 adults with TTP, although patients randomized to the plasma exchange arm received more plasma. The exchange of 1 plasma volume daily is standard initial treatment. Plasma exchange is continued daily until the platelet count reaches normal levels and symptoms have resolved. Neurologic symptoms improve most rapidly. No evidence suggests a benefit of either abrupt discontinuation or tapering of plasma exchange. Antiplatelet agents have not been shown to be beneficial and may increase bleeding, although some guidelines advocate their use in patients in whom the platelet count increases rapidly during plasma exchange. Corticosteroids are used initially in most patients with TTP because of the presence of ADAMTS13 antibodies, although a significant benefit has not been demonstrated consistently in randomized studies. In recent years, the potential utility of rituximab in TTP has been revealed. In a single-arm study, the addition of rituximab in patients who did not respond rapidly to plasma exchange led to more rapid resolution of TTP and a lower incidence of relapse compared with historical controls. Other studies have demonstrated the apparent efficacy of rituximab in relapsed TTP and the disappearance of ADAMTS13 antibodies following treatment. Other adjunctive therapies for refractory TTP include immunosuppressive agents, such as cyclosporine and vinristine, as well as splenectomy, which may decrease relapse rates. Platelet transfusion has been associated with a rapid decline in clinical status in occasional patients and is relatively contraindicated. Caplacizumab is an anti-VWF nanobody under development for treatment of TTP. It has not been approved by the FDA. In a phase III trial, caplacizumab added to plasma exchange shortened the time to platelet response and reduced the composite outcome of TTP-related death, TTP recurrence, and major thromboembolic events compared with plasma exchange alone.

Response rates to plasma exchange in patients with aHUS are not as robust as in TTP. Eculizumab, an antibody against complement C5, has shown efficacy in patients with aHUS, leading to its approval for aHUS treatment in 2011. Delays in initiation of eculizumab are associated with worse long-term renal function and a greater likelihood of dialysis-dependence. Therefore, eculizumab should be initiated promptly in patients with TMA who do not have severe deficiency of ADAMTS13, Shiga toxin-producing *E. coli*, or another apparent cause of TMA and who do not respond to plasma exchange. Treatment should not be delayed until complement mutation results are available because turnaround time for this testing is several weeks and because some patients

with clinical aHUS without identifiable mutations benefit from eculizumab. The current standard of care is to continue eculizumab indefinitely in patients with aHUS, though mounting evidence suggests that it may be safe to discontinue treatment (with close surveillance) in selected patients.

Treatment of *E. coli*-associated typical HUS is generally supportive. It was long assumed that the use of antibiotics may lead to increased toxin release and worse outcome. However, during an epidemic outbreak of typical HUS, antibiotic treatment was associated with reduced morbidity. Some patients may require transfusion support and/or dialysis during the acute phase of their illness. A benefit for plasma exchange in typical HUS has not been demonstrated. Immunoabsorption using anti-IgG columns resulted in rapid reversal of severe neurological symptoms and normalization of renal function in patients with *E. coli* O104:H4-associated HUS.

KEY POINTS

- TTP, aHUS, and typical (Shiga-like toxin; Stx) HUS share many common features and may be difficult to distinguish from one another.
- The pathogenesis of TTP involves deficiency of ADAMTS13, usually because of acquired autoantibodies against ADAMTS13. This leads to accumulation of ultralarge VWF multimers that induce platelet aggregation in the microcirculation.
- aHUS involves excessive activation of the AP, leading to complement-mediated damage to vascular cells.
- The pathogenesis of typical HUS reflects the effects of Shiga toxin on vascular endothelium and other cell types.
- The treatment of choice for acquired TTP is plasma exchange, often supplemented with corticosteroids and rituximab.
- Plasma exchange is effective in some cases of aHUS. Eculizumab should be used in patients who do not respond promptly to plasma exchange.
- Plasma exchange is not effective in typical HUS, which is usually self-limited. Treatment is supportive.

Splenic sequestration

Splenic enlargement, most commonly from cirrhosis and portal hypertension, results in sequestration of platelets in the splenic vascular network, leading to mild to moderate thrombocytopenia. Typical platelet counts in patients with splenic sequestration are 60 to $100 \times 10^9/L$. Other mechanisms associated with liver disease that may induce thrombocytopenia include hepatitis C-induced secondary

ITP, suppression of platelet production by megakaryocytes resulting from direct viral infection, and decreased production of TPO by the cirrhotic liver.

Hereditary thrombocytopenia

Hereditary thrombocytopenic syndromes are uncommon but not as rare as once assumed. It is critical that treating physicians maintain a high index of suspicion for these disorders, as patients are often misdiagnosed as having ITP, resulting in unnecessary, ineffective, and potentially harmful treatments such as immunosuppression and splenectomy. In about 50% of affected families, at least 1 family member has been splenectomized to treat “ITP.” The diagnosis should be considered in any patient with a family history of thrombocytopenia, in patients with long-lasting “ITP” who do not respond to standard therapy, or when there is bleeding out of proportion to the degree of thrombocytopenia (eg, an intracranial hemorrhage in an “ITP” patient with a platelet count of $60 \times 10^9/L$). Whenever possible, physicians should attempt to document a historical normal platelet count in a patient with thrombocytopenia to exclude a hereditary thrombocytopenic disorder. The presence of anatomic defects, including absent radii (thrombocytopenia-absent radius [TAR] syndrome) or right-heart defects (22q11.2 deletion syndrome), high-tone hearing loss, cataracts before age 50, or interstitial nephritis support the diagnosis of hereditary thrombocytopenia. The blood smear is also essential for identifying patients with potential hereditary thrombocytopenia. For example, large platelets and neutrophil inclusions may indicate the presence of a *MYH9*-related disorder. Genetic testing may be used to confirm the diagnosis. Although about 40% of families with inherited thrombocytopenia do not have an identifiable gene defect, this is a rapidly evolving area and the advent of next-generation sequencing has expanded the phenotypes of some of the classical platelet disorders.

Thrombocytopenia with large platelets

Many inherited thrombocytopenias involve defects in platelet production, while megakaryocytopoiesis is largely normal. The platelet mass is distributed to fewer platelets, which results in macrothrombocytopenia. Automated particle counters often underestimate the platelet number by counting the large platelets as red cells or leukocytes. While platelet size may be increased in ITP or myeloproliferative neoplasms (MPNs), the platelet population is typically heterogeneous with large- and normal-sized platelets. Any blood smear showing >60% large platelets is highly suspicious for a hereditary macrothrombocytopenia.

The most common of the macrothrombocytopenias, *MYH9*-related thrombocytopenia, is an autosomal dominant macrothrombocytopenia that formerly consisted of the May-Hegglin, Fechtner, Sebastian, and Epstein syndromes. All of these are caused by variants in the *MYH9* gene, which codes for nonmuscle myosin IIA. In addition to macrothrombocytopenia, the peripheral blood film typically demonstrates Döhle body-like inclusions in neutrophils (which are best detected by immunofluorescence, Figure 11-4). Associated clinical features including hearing loss, cataracts, and renal failure are present in some patients. Bleeding symptoms are mild to moderate because platelet function is nearly normal apart, from a reduction in platelet cytoskeleton contraction with resulting reduced clot stability. About 30% of patients have a de novo mutation and therefore a negative family history. Large platelets are also found in a subgroup of VWD type IIB (Montreal platelet disorder) and in both monoallelic and biallelic Bernard-Soulier syndrome (BSS), which is characterized by the decreased expression of the platelet GPIb-IX complex, lack of platelet agglutination with high-dose ristocetin, and bleeding (see the section “Disorders of platelet function”). Furthermore, variants in the platelet cytoskeleton proteins beta tubulin (TUBB1), filamin (FLNA), alpha actinin (ACTN1), tropomyosin 4 (TPM4), and DIAPH1, a member of the formin family which regulates microtubule assembly, all result in macrothrombocytopenia. DIAPH1 variants are also associated with sensorineural hearing loss. More severe bleeding disorders associated with macrothrombocytopenia are seen in biallelic BSS, *PRKACG*-related thrombocytopenia, and in patients with activating variants in *ITGA2B/ITGB3*, which cause Glanzmann thrombasthenia.

Hereditary macrothrombocytopenias also occur in association with mutations in specific transcription factors that regulate megakaryocyte and platelet production, including *GATA1* (X-linked inheritance, dyserythropoiesis) (see “Disorders of platelet function” for more information). Patients with the Paris-Trousseau/Jacobsen syndrome, an autosomal dominant macrothrombocytopenia, have psychomotor retardation and facial and cardiac abnormalities. This syndrome arises because of deletion of a portion of chromosome 11, 11q23–24, that encompasses the gene encoding the transcription factor friend leukemia integration 1 (FLI1). Autosomal recessive inheritance of variants in this gene alone reproduce the Paris-Trousseau platelet phenotype without the associated cardiac and developmental abnormalities. Gray platelet syndrome (deficiency of alpha granules) results from variants in the *NBEAL2* gene (recessive trait) and generally causes a macrothrombocytopenia. Variants in GFI1b have been shown to cause

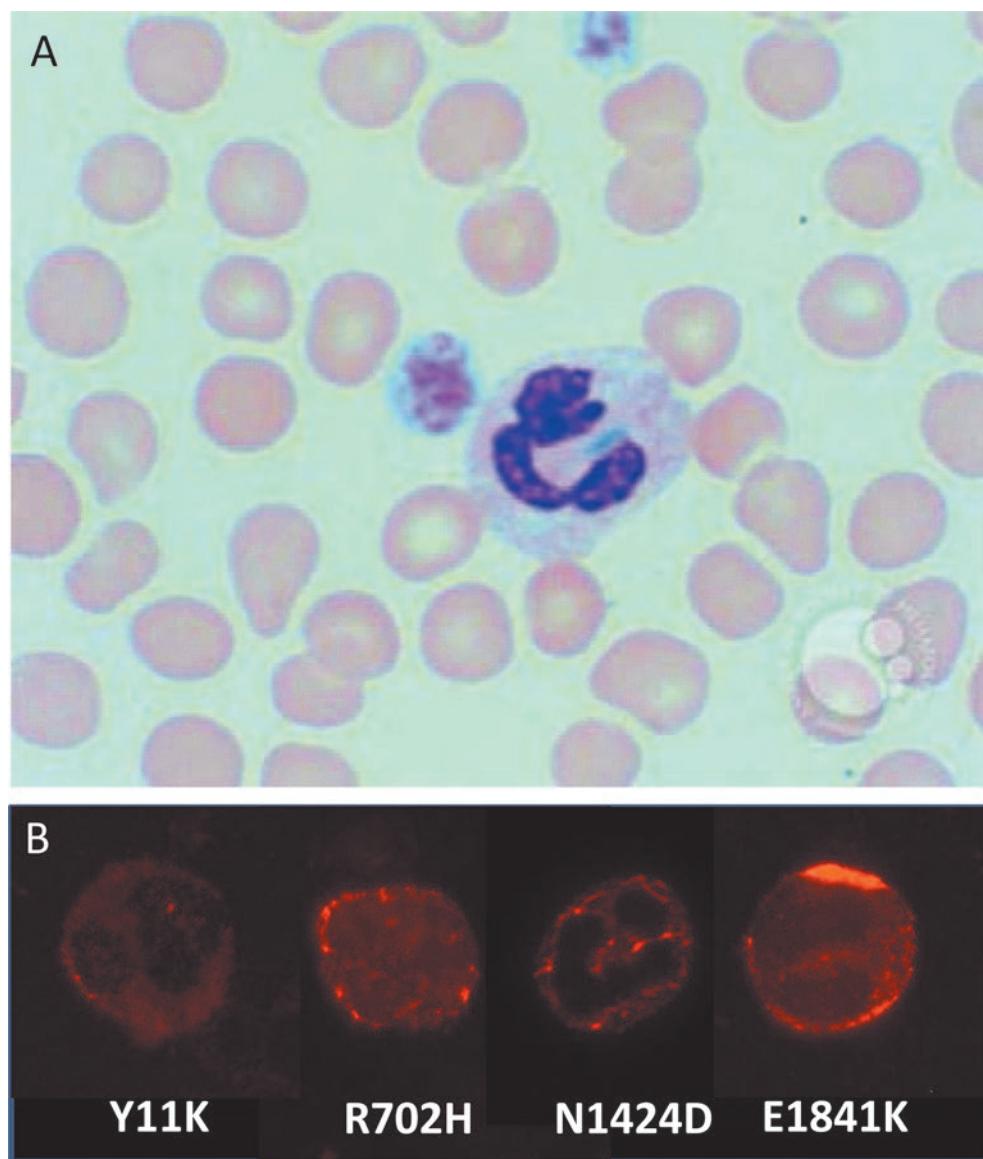


Figure 11-4 MYH9-associated macrothrombocytopenia. Heterozygous mutations in the *MYH9* gene encoding nonmuscular myosin IIa are the most frequent cause of hereditary macrothrombocytopenia. The mutated protein clusters in the cytoplasm of neutrophils. The inclusion bodies differ in size and shape depending on the mutation. Large inclusion bodies result from mutations in the downstream part of the gene and are visible by both light microscopy and immunofluorescence. More upstream mutations result in smaller inclusion bodies that are visible only with immunofluorescence. (A) Peripheral blood film from a patient with a downstream mutation. A giant platelet is visible. Adjacent to the platelet is a neutrophil containing a large blue Döhle-like body. Source: ASH Image Bank/Julie Braza. (B) Immunofluorescence stain from patients with 4 different mutations.

a platelet defect that is similar to gray platelet syndrome, with loss of platelet granules and variable alterations in platelet function inherited in an autosomal dominant fashion. Activating mutations in *SRC* cause a juvenile myelofibrosis-associated thrombocytopenia inherited in an autosomal dominant fashion.

Thrombocytopenia with normal-sized platelets

Normal-sized platelets are found in 3 autosomal dominant, inherited thrombocytopenias with associated increased risk of myeloid malignancy: *RUNX1* (with variable platelet dysfunction and therefore variable bleeding), *ANKRD26* (mild to no bleeding), and *ETV6* (mild to no

bleeding). The risk of malignancy with these disorders is markedly increased. With *RUNX1* defects, the thrombocytopenia is not 100% penetrant. Therefore, genetic screening should include even those family members with normal platelet counts. *RUNX1* variants are also discussed in the section “Disorders of platelet function.” The inherited thrombocytopenias associated with increased risk of bone marrow failure are also generally associated with normal platelet size: congenital amegakaryocytic thrombocytopenia (CAMT), TAR, radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT). CAMT, a recessive disorder due to mutations in the c-Mpl receptor, is characterized by severe thrombocytopenia, absence of megakaryocytes in the bone marrow, and a risk of trilineage failure. TAR syndrome is inherited in a compound fashion, with most patients coinheriting a microdeletion of 1q21 encompassing the *RBM8A* gene and 1 of 2 polymorphisms on the other chromosome in *RBM8A* associated with decreased expression. RUSAT results from autosomal dominant inheritance of *HOXA11* variants or autosomal recessive variants in *MECOM*. The autosomal recessive form is associated with an increased risk of bone marrow failure and myelodysplastic syndrome.

Small platelets are typical of Wiskott-Aldrich syndrome (WAS), an X-linked disorder characterized by severe immunodeficiency, small platelets, and eczema (this is described in more detail in the section “Disorders of platelet function”). Two additional autosomal recessive disorders with small platelets have been recently described: *FYB*-related thrombocytopenia with isolated small platelets and thrombocytopenia and a very rare disorder of inflammation, eosinophilia and microthrombocytopenia due to variants in *ARPC1*.

Establishing the diagnosis of hereditary thrombocytopenia may be difficult. Historically, demonstration of decreased expression of platelet GPIb-IX using flow cytometry has been used to diagnose BSS. Clustering of myosin in granulocytes using an immunofluorescent antibody against nonmuscle myosin heavy chain type IIa may aid in screening for *MYH9*-related disorders. Improvements in sequencing technologies have allowed for the expansion of genetic analyses for BSS, *MYH9*-related thrombocytopenia, CAMT, *GATA1*-related thrombocytopenia, TAR syndrome, and WAS-associated thrombocytopenia. Several laboratories in the United States and Europe now provide these services (see <http://www.genetests.org>). Expanded genetic panels include some of the novel inherited thrombocytopenias, allowing for diagnosis in many families, but up to 40% of families still do not carry molecular diagnosis.

KEY POINTS

- Splenic sequestration is a common cause of thrombocytopenia in patients with liver disease.
- Failure to respond to standard ITP therapy (corticosteroids, IVIg) should prompt consideration of a hereditary thrombocytopenia.
- Genetic diagnosis of hereditary thrombocytopenia should be obtained when possible.

Infection-associated thrombocytopenia and hemophagocytic lymphohistiocytosis

Mild and transient thrombocytopenia occurs with many systemic infections. Thrombocytopenia may be caused by a combination of mechanisms, including decreased platelet production, increased destruction, and increased splenic sequestration. In viral infections, infection of megakaryocytes may lead to suppression of platelet production; in rickettsial infections, platelets may be consumed in vasculitic lesions; in bacteremia, platelet consumption may result from DIC or enhanced clearance of immune complex-coated platelets. HIV, hepatitis C virus, and *H. pylori* are causes of secondary ITP. A rare and unusual cause of infection-related thrombocytopenia is the hemophagocytic syndrome, also known as hemophagocytic lymphohistiocytosis (HLH). This disorder may be inherited, occur in conjunction with rheumatologic disease or malignancy, or occur in response to infection, with Epstein-Barr virus (EBV) being the most common associated pathogen. HLH is more common in children and is characterized by persistent activation of macrophages and cytotoxic T cells, leading to damage of multiple organ systems. Thrombocytopenia occurs in most patients, usually in the context of bycytopenia or pancytopenia. Characteristic clinical and laboratory features in addition to cytopenias include fever (due to hypercytokinemia), hepatosplenomegaly (due to accumulation of macrophages), elevated triglyceride levels (due to inhibition of lipoprotein lipase by elevated tumor necrosis factor-alpha levels), elevated ferritin levels, elevated levels of the circulating α-chain of the interleukin-2 (IL-2) receptor (due to increased secretion from macrophages), low fibrinogen levels (due to release of tissue plasminogen activator from activated monocytes), and low or absent natural killer cell activity. Treatment of HLH includes suppression of hyperinflammation and reduction of activated cells by immunosuppressive therapy. Demonstration of hemophagocytosis on tissue or bone marrow biopsies is useful but not

required. In cases associated with EBV, therapy is directed toward eradication of EBV-infected cells.

Thrombocytopenia in the critically ill

This topic is covered in greater detail in Chapter 2. Approximately 40% of patients in medical or surgical intensive care units (ICUs) develop a platelet count $<150 \times 10^9/L$; 20% to 25% develop a platelet count $<100 \times 10^9/L$; and 12% to 15% develop severe thrombocytopenia with a platelet count $<50 \times 10^9/L$. The development of thrombocytopenia in patients in the ICU is a strong independent predictor of mortality. The spectrum of disorders that cause thrombocytopenia in this setting is extensive and includes DITP, infection, DIC, surgery, hemodilution, extracorporeal circuitry/intravascular devices (eg, cardiopulmonary bypass, intra-aortic balloon pumps, extracorporeal membrane oxygenation), and HIT, among others. Management is highly dependent on the etiology of thrombocytopenia. For example, whereas platelet transfusion and suspension of anticoagulant prophylaxis may be indicated in a patient with DITP, HIT requires prompt initiation of an alternative nonheparin anticoagulant and constitutes a relative contraindication to platelet transfusion. Treatment must therefore be individualized to the underlying cause of thrombocytopenia and any concomitant hemorrhagic or thrombotic risk factors the patient may harbor. High-quality evidence linking a platelet count threshold with bleeding risk in ICU patients is lacking. Prophylactic platelet transfusion is generally given when the platelet count decreases to 10×10^9 to $20 \times 10^9/L$. Patients with bleeding or a planned invasive procedure may require a higher platelet count.

KEY POINTS

- Infection is a common cause of thrombocytopenia, particularly in ICU patients, and can be induced by a variety of organisms.
- Thrombocytopenia in the ICU may arise from a number of etiologies. Treatment should be individualized based on the etiology of thrombocytopenia and individual patient factors.

Disorders of platelet function

Disorders of platelet function (see video in online edition) are characterized by variable mucocutaneous bleeding manifestations and excessive hemorrhage following surgical procedures or trauma. Spontaneous hemarthrosis and deep hematomas are unusual in patients with platelet defects. Intracranial hemorrhage is also rare, but can occur. Most patients have mild to moderate bleeding manifesta-

tions. Platelet aggregation and secretion studies provide evidence for the defect, but generally are not predictive of the severity of clinical manifestations. Defects in platelet function may be inherited or acquired, with the latter being far more commonly encountered.

Inherited disorders of platelet function

CLINICAL CASE



A 9-year-old girl is referred by her pediatrician for evaluation of long-standing easy bruising and recurrent epistaxis. She has not had any surgery. The physical examination reveals scattered bruises on the lower extremities. The platelet count is $190 \times 10^9/L$, and the hemoglobin is 11 g/dL. Plasma levels of factor VIII, VWF antigen, and ristocetin cofactor are within normal range. The hematologist recommends platelet aggregation studies. These studies reveal abnormal platelet aggregation responses upon activation—a primary wave but no secondary wave in response to ADP and epinephrine and decreased aggregation with collagen. The response to ristocetin is normal. The patient is diagnosed with a platelet secretion defect.

Table 11-6 provides a classification of inherited disorders associated with impaired platelet function (Figure 11-1). Of note, not all of these disorders are due to a defect in the platelet per se. Some, such as VWD and afibrinogenemia, result from deficiencies of plasma proteins essential for platelet adhesion or aggregation. Some of these disorders are distinctly rare but shed light on platelet physiology. In the majority of patients with inherited abnormalities of platelet function, the molecular defect remains unknown, suggesting that some of these disorders may be the result of coinheritance of multiple hypofunctional variants. In patients with defects in platelet–vessel wall interactions (adhesion disorders), adhesion of platelets to subendothelium is abnormal. There are 4 disorders in this group: VWD (resulting from a deficiency or abnormality in plasma VWF), platelet-type VWD (resulting from variants in GPIb that more avidly bind VWF resulting in loss of high-molecular-weight multimers), BSS (where platelets are deficient in the GPIb-IX complex resulting in impairment of platelet–VWF interaction), and platelet deficiency of GPVI (impaired binding of platelets to the subendothelium due to decreased expression of GPVI, the collagen receptor on platelets). Binding of fibrinogen to the GPIIb-IIIa complex is a prerequisite for platelet aggregation. Disorders characterized by abnormal platelet–platelet interactions (aggregation disorders) arise because of a severe deficiency of plasma fibrinogen (congenital

afibrinogenemia) or because of a quantitative or qualitative abnormality of the platelet membrane GPIIb-IIIa complex, which binds fibrinogen (Glanzmann thrombasthenia). Two variant forms of Glanzmann thrombasthenia have been described. Patients with variants in *FERMT3* have leukocyte adhesion deficiency type III with abnormal wound healing, increased infections, and a Glanzmann-like platelet function defect with severe bleeding presenting in infancy. Patients with variants in *RASGRP2* present early in life with similarly significant bleeding due to inability to fully activate GPIIb-IIIa, resulting in abnormal platelet aggregation. The remainder of the platelet function defects are grouped according to the mechanism by which platelet dysfunction occurs, but many of these disorders have not yet been fully molecularly characterized. Those that have are often associated with other clinical manifestations due to effects of the variants on pathways outside the platelet. Patients with defects in platelet secretion and signal transduction are a heterogeneous group lumped together for convenience of classification rather than an understanding of the specific underlying abnormality. The major common characteristics in these patients, as currently perceived, are abnormal aggregation responses and an inability to release intracellular granule (dense) contents upon activation of platelet-rich plasma with agonists such as ADP, epinephrine, and collagen. In aggregation studies, the second wave of aggregation is blunted or absent.

The patient described in the clinical case at the beginning of this section falls in this heterogeneous group of “platelet secretion defects.” The platelet dysfunction may arise from a variety of mechanisms. A small proportion of these patients have a deficiency of dense granule stores (storage pool deficiency). In other patients, the impaired secretion results from aberrations in the signal transduction events or in pathways leading to thromboxane synthesis that govern end-responses, such as secretion and aggregation. The findings on aggregation studies are non-specific, and it is not possible to pinpoint a specific molecular abnormality from the tracings. Another group consists of patients who have an abnormality in interactions of platelets with proteins of the coagulation system; the best described is the Scott syndrome, which is characterized by impaired transmembrane migration of procoagulant-phospholipid during platelet activation. Defects related to platelet cytoskeletal or structural proteins also may be associated with platelet dysfunction, often on the basis of impaired signal transduction or platelet granule secretion because of abnormal interactions between cytoskeleton and membrane. Finally, variants in transcription factors (eg, RUNX1, GATA1, FLI1 and GFI1b) that regulate the expression of important platelet proteins may also vari-

ably impact platelet function because of abnormal granule packaging. The prevalence and relative frequencies of the various platelet abnormalities remain unknown.

Disorders of platelet adhesion

Bernard-Soulier syndrome

BSS, a rare autosomal recessive platelet function disorder, results from an abnormality in the platelet GPIb-IX complex, which mediates the binding of VWF to platelets and thus plays a major role in platelet adhesion to the subendothelium, especially at high shear rates. GPIb exists in platelets as a complex consisting of GPIb, GPIX, and GPV. There are approximately 25,000 copies of GPIb-IX on the surface of an individual platelet, and these are reduced or abnormal in BSS. Although GPV also is decreased in BSS platelets, it is not required for platelet surface GPIb-IX expression. The platelet count is moderately decreased, and platelets are markedly increased in size on the peripheral smear. In platelet aggregation studies, responses to ADP, epinephrine, thrombin, and collagen are normal and response to ristocetin is decreased or absent, a feature shared with severe VWD. This is because ristocetin-induced platelet agglutination is mediated by binding of VWF to the GPIb complex. Unlike in VWD, however, plasma VWF and factor VIII are normal in BSS, and the addition of normal donor VWF does not restore ristocetin-induced agglutination of platelets because of GPIb deficiency on the patient’s platelets. Dense granule secretion on activation with thrombin may be decreased in these patients. The diagnosis of BSS is established by demonstrating decreased platelet surface GPIb, which can be performed using flow cytometry. The most severe phenotype is associated with the biallelic form of BSS where variants are inherited from both parents, resulting in markedly reduced expression and/or function of the GPIb-IX complex. Monoallelic forms (autosomal dominant BSS, benign Mediterranean macrothrombocytopenia, Bolzano variant of BSS) have been described as well, with a less severe phenotype and decreased expression of GPIb-IX and variable response to ristocetin on platelet function testing.

von Willebrand disease

See the section titled “von Willebrand disease” in Chapter 10.

Disorders of platelet aggregation

Glanzmann thrombasthenia

Glanzmann thrombasthenia is a rare autosomal recessive disorder characterized by markedly impaired platelet aggregation and relatively severe mucocutaneous bleeding

manifestations compared with most other platelet function disorders. It has been reported in clusters in populations in which consanguinity is common. Normal resting platelets possess approximately 50,000 to 80,000 GPIIb-IIIa complexes on their surface. The primary abnormality in Glanzmann thrombasthenia is a quantitative or qualitative defect in the GPIIb-IIIa complex, a heterodimer consisting of GPIIb and GPIIIa whose synthesis is governed by distinct genes located on chromosome 17. Thus, thrombasthenia may arise due to a mutation in either gene, with decreased platelet expression of the complex. Platelet aggregation is mediated through interaction of GPIIb-IIIa and fibrinogen. In Glanzmann thrombasthenia, platelet aggregation is impaired. Clot retraction, a function of the interaction of GPIIb-IIIa with the platelet cytoskeleton, is also compromised.

Binding of fibrinogen to the GPIIb-IIIa complex on platelet activation is required for aggregation in response to all physiologic agonists. Thus, the diagnostic hallmark of thrombasthenia is the absence or marked decrease of platelet aggregation in response to all platelet agonists (except ristocetin), with absence of both the primary and secondary wave of aggregation. The shape change response is preserved. Platelet-dense granule secretion may be decreased with weak agonists (eg, ADP) but is normal on activation with thrombin. Heterozygotes have approximately half the number of platelet GPIIb-IIIa complexes, but platelet aggregation responses are normal. Although congenital afibrinogenemia is also characterized by absence of platelet aggregation, it can be distinguished from thrombasthenia by a prolonged PT, aPTT, and thrombin time and absent fibrinogen. The diagnosis of thrombasthenia can be confirmed by demonstrating decreased platelet expression of the GPIIb-IIIa complex using flow cytometry. A subgroup of disorders of the GPIIb-IIIa complex are inherited dominantly. These patients have moderately decreased platelet numbers and large platelets. The underlying cause is a mutation in the transmembrane or intracellular part of the integrin, which results in permanent activation of the GPIIb-IIIa complex. Finally, recently described defects in genes downstream of the integrin complex in *FERMT3* and *RASGRP2* result in Glanzmann-like platelet function defects with severe bleeding phenotype and markedly abnormal platelet responses by light transmission aggregometry, but only mild or moderate decrease in expression of GPIIb-IIIa on the surface of platelets.

Disorders of platelet secretion and signal transduction

As a unifying theme, patients lumped into this remarkably heterogeneous group generally are characterized by

impaired dense granule secretion and the absence of a second wave of aggregation upon stimulation of platelet-rich plasma with ADP or epinephrine; responses to collagen, thromboxane analog (U46619), and arachidonic acid also may be impaired. Conceptually, platelet function is abnormal in these patients either because the granule contents are diminished (storage pool deficiency) or because the mechanisms mediating or potentiating aggregation and secretion are impaired (Table 11-6). Identification of the underlying defect is often very difficult and in many patients it is caused by a combination of several minor abnormalities.

Defects of granule biogenesis

Many of the defects of granule biogenesis result in a common phenotype called storage pool deficiency (SPD). SPD refers to deficiency in platelet content of dense granules (δ -SPD), α -granules (α -SPD), or both types of granules ($\alpha\delta$ -SPD). Often, the platelet phenotype in these disorders is part of a broader syndromic disease and can be recognized by the other systemic manifestations.

Patients with δ -SPD have a mild to moderate bleeding diathesis. In platelet studies, the second wave of aggregation in response to ADP and epinephrine is absent or blunted, and the collagen response is markedly impaired. Normal platelets possess 3 to 8 dense granules (each 200 to 300 nm in diameter). Under the electron microscope, dense granules are decreased in δ -SPD platelets. By direct biochemical measurements, the total platelet and granule ATP and ADP contents are decreased along with other dense granule constituents including calcium, pyrophosphate, and serotonin.

δ -SPD has been reported in association with other inherited disorders, such as Hermansky-Pudlak syndrome (HPS) (oculocutaneous albinism and increased reticuloendothelial ceroid), Chediak-Higashi syndrome, and Griscelli syndrome. Some patients with WAS have been reported to manifest δ -SPD as part of their platelet phenotype. The simultaneous occurrence of δ -SPD and defects in skin pigment granules, as in HPS, point to commonalities in the pathways that govern synthesis and trafficking of platelet-dense granules and melanosomes.

In northwest Puerto Rico, HPS affects 1 of every 1,800 individuals. There are at least 9 known HPS-causing genes, with most patients having HPS-1 and being from Puerto Rico. HPS is autosomal recessive and heterozygotes classically have no clinical findings. In addition to albinism, many patients have congenital nystagmus and decreased visual acuity. Two additional manifestations associated with certain HPS subtypes are granulomatous colitis and pulmonary fibrosis. With next generation sequencing, the

Table 11-6 Inherited disorders of platelet function

1. Defects in platelet–vessel wall interaction (disorders of adhesion)
a. von Willebrand disease (deficiency or defect in plasma VWF)
b. Bernard–Soulier syndrome (deficiency or defect in GPIb)
c. GPVI deficiency
2. Defects in platelet–platelet interaction (disorders of aggregation)
a. Congenital afibrinogenemia (deficiency of plasma fibrinogen)
b. Glanzmann thrombasthenia (deficiency or defect in GPIIb–IIIa)
B1: <i>FERMT3</i> variants causing LAD-III (GT-like platelet defect with immunodeficiency, poor wound healing, +/- osteopetrosis)
B2: <i>RASGRP2</i> variants causing deficiency of GαDAG–GEFI and abnormal signaling/activation of GPIIb/IIIa and GT-like platelet defect
3. Disorders of granule biogenesis
a. δ-SPD (isolated, HPS, CHS, Griscelli)
b. α-SPD (GPS, ARC, QPS)
4. Disorders of platelet secretion and signal transduction
a. Receptor defects (TXA2R, P2Y12, F2RL3, P2RX1, GP6)
b. G-protein (Gαq, Gαs, Gαi) abnormalities
c. Defects in phosphatidylinositol metabolism and protein phosphorylation
• Phospholipase C-β2 deficiency
• PKC-θ deficiency
d. Abnormalities in arachidonic acid pathways and thromboxane A ₂ synthesis
• Phospholipase A ₂ deficiency
• Cyclooxygenase deficiency
• Thromboxane synthase deficiency
e. Defects in signal transduction
• <i>RASGRP2</i> (CalDAG-GEF1)
• <i>FERMT3</i> (kindlin-3)
• <i>PRKACG</i>
5. Disorders of platelet coagulant–protein interaction (Scott syndrome)
Stormorken/York platelet syndrome (increased baseline PS, abnormal calcium flux)
6. Defects related to cytoskeletal/structural proteins
a. Wiskott–Aldrich syndrome
b. Filamin deficiency (<i>FLNA</i>) (<i>TUBB1</i> , <i>ACTN1</i> , <i>MYH9</i> not typically associated with significant platelet dysfunction)
7. Abnormalities of transcription factors leading to functional defects
a. <i>RUNX1</i>
b. <i>GATA1</i>
c. <i>FLI1</i> (Paris–Trousseau platelets, abnormal function)
d. <i>GFI1B</i>

Modified with permission from Rao AK. *Am J Med Sci*. 1998;316:69–77.
GATA1, sex-linked inheritance; RUNX1, autosomal dominant.

phenotype for some HPS variants is expanding and now also includes neutropenia and immunodeficiency.

Chediak–Higashi syndrome is a rare autosomal recessive disorder characterized by δ-SPD, oculocutaneous albinism, immune deficiency, cytotoxic T and natural killer

cell dysfunction, neurologic symptoms, and the presence of giant cytoplasmic inclusions in different cells. It arises from mutations in the lysosomal trafficking regulator (*LYST*) gene on chromosome 1.

Patients with gray platelet syndrome (GPS) have an isolated deficiency of platelet α-granule contents. The name refers to the initial observation that the platelets have a gray appearance with a paucity of granules on the peripheral blood smear. These patients have a bleeding diathesis, mild thrombocytopenia, and a prolonged bleeding time. The inheritance pattern is variable; autosomal recessive, autosomal dominant, and sex-linked patterns have been described. Classical GPS (autosomal recessive) appears to be due to variants in the *NBEAL2* gene. The autosomal dominant form is associated with variants in *GFI1b* and red cell anisocytosis while the X-linked form has been associated with variants in *GATA1*. Finally, in patients with arthrogryposis–renal dysfunction–cholestasis (ARC) syndrome, caused by variants in *VPS33B* or *VI-PAS39*, there is low α-granule content and platelet dysfunction in a setting of fairly severe systemic disease that is often lethal in early childhood. In all of these disorders, under the electron microscope, platelets and megakaryocytes reveal absent or markedly decreased α-granules. The platelets are severely and selectively deficient in α-granule proteins including PF4, βTG, VWF, thrombospondin, fibronectin, factor V, and platelet-derived growth factor. In some patients, plasma PF4 and βTG are raised, suggesting that the defect is not in their synthesis by megakaryocytes but rather in their packaging into granules. Platelet aggregation responses are variable. Responses to ADP and epinephrine are normal in most patients; in some patients, aggregation responses to thrombin, collagen, and ADP are impaired.

The Quebec platelet disorder is an autosomal dominant disorder associated with delayed bleeding and abnormal proteolysis of α-granule proteins (including fibrinogen, factor V, VWF, thrombospondin, multimerin, and P-selectin) resulting from increased amounts of platelet urokinase-type plasminogen activator (uPA). Patients have normal to reduced platelet counts, proteolytic degradation of α-granule proteins, and a defective aggregation response with epinephrine. This disorder is caused by tandem duplication of a 78-kb region of chromosome 10 containing *PLAU* (the uPA gene), a mechanism unique among inherited platelet disorders.

Defects in platelet signal transduction and platelet activation

Signal transduction mechanisms encompass processes that are initiated by the interaction of agonists with specific

platelet receptors and include responses such as G-protein activation and activation of phospholipase C and phospholipase A₂ (Figure 11-1). Patients with disorders of platelet signal transduction and activation present with variable bleeding ranging from mild platelet-type bleeding (similar to that seen in patients treated with antiplatelet therapies) to more severe bleeding, depending on the degree of impairment of platelet function.

Patients with receptor defects have impaired responses because the platelet surface receptors for a specific agonist are decreased. Such defects have been documented in the P2Y12 ADP receptor (the receptor targeted by thienopyridines such as clopidogrel), TxA₂ receptor, collagen receptors (GPIa-IIa and GPVI), and epinephrine receptor. Because ADP and TxA₂ play a synergistic role in platelet responses to several agonists, patients with defects in the receptors for these agonists manifest abnormal aggregation responses to multiple agonists.

G proteins serve as a link between surface receptors and intracellular effector enzymes and defects in G protein (G α q, G α i, and G α s) activation can impair platelet signal transduction. As G proteins are required in many cell types, affected patients typically have syndromic phenotypes, often associated with neural deficiencies.

Downstream abnormalities in platelet function have also been identified, such as defects in phospholipase C activation (phospholipase C- β 2 and PKC- θ), calcium mobilization, and pleckstrin phosphorylation. A major platelet response to activation is liberation of arachidonic acid from phospholipids and its subsequent oxygenation to TxA₂, which plays a synergistic role in the response to several agonists. Patients have been described with impaired thromboxane synthesis because of congenital deficiencies of phospholipase A₂, cyclooxygenase, and thromboxane synthase.

Disorders of platelet procoagulant activities

Platelets play a major role in blood coagulation by providing the surface on which several specific key enzymatic reactions occur. In resting platelets, there is an asymmetry in the distribution of some of the phospholipids such that phosphatidylserine and phosphatidylethanolamine are located predominantly on the inner leaflet, whereas phosphatidylcholine has the opposite distribution. Platelet activation results in a redistribution with expression of phosphatidylserine on the outer surface, mediated by phospholipid scramblase. The exposure of phosphatidylserine on the outer surface is an important event in the expression of platelet procoagulant activities. Rare patients have been described in whom this process is impaired, and this is referred to as Scott syndrome. In these patients,

who have a bleeding disorder, the bleeding time and platelet aggregation responses are normal. Recently, a second group of platelet disorders has been associated with abnormally increased expression of phosphatidylserine at baseline and increased intracellular platelet Ca²⁺ levels due to abnormal function of the Ca²⁺ release-activated Ca²⁺ channels formed by a pore protein (ORAI1) and Ca²⁺ sensor STIM1. Variants in either *ORAI1* or *STIM1* (activating mutations) have been associated with Stomarken syndrome/York platelet syndrome, a group of disorders characterized by bleeding, abnormal platelets, and myopathy.

Other abnormalities

Platelet function abnormalities have been described in association with other entities such as WAS, GATA1, and FLI1. More recently, abnormal platelet function has been documented in patients with mutations in transcription factor RUNX1, which results in dysregulation of platelet biogenesis in general and abnormal expression of multiple platelet genes including *GATA1*, *MYH9*, *NFE2*, *MYL9*, and *PKC*. Patients with RUNX1 mutations have hereditary thrombocytopenia, platelet dysfunction, and predisposition to acute leukemia.

Treatment of inherited platelet function defects

Because of the wide disparity in bleeding manifestations, management needs to be individualized. Platelet transfusions are indicated in the management of significant bleeding and in preparation for surgical procedures. Platelet transfusions are effective in controlling bleeding manifestations but come with potential risks associated with blood products, including alloimmunization in patients lacking platelet GPs. For example, patients with Glanzmann thrombasthenia and BSS may develop alloantibodies against GPIb-IIIa and GPIb, respectively, which compromise the efficacy of subsequent platelet transfusions. An alternative to platelet transfusions is administration of desmopressin (DDAVP), which shortens the bleeding time in some patients with platelet function defects, depending on the platelet abnormality. Most patients with thrombasthenia do not show a shortening of the bleeding time following DDAVP infusion, whereas responses in patients with signaling or secretory defects are variable. Recombinant factor VIIa has been approved for the management of bleeding events in patients with Glanzmann thrombasthenia and has been used in some other inherited defects. Hormonal contraceptives and/or antifibrinolytic therapy are often effective for management of menorrhagia. Other minor mucosal bleeding may respond to intranasal DDAVP or antifibrinolytic agents. Bone marrow trans-

plant is being used increasingly in the most severe platelet disorders (WAS, Glanzmann thrombasthenia) and successful gene therapy trials suggest this may also be an option in some disorders (eg, WAS).

A basic therapeutic principle in all patients with platelet disorders is to prevent iron-deficiency anemia. Red blood cells are required for sufficient hemostasis. Iron-deficiency anemia is common in this population, especially in women of childbearing age. Oral iron supplementation may be insufficient to normalize iron stores, and intravenous iron therapy may be required.

KEY POINTS

- Patients with inherited platelet defects typically have mucocutaneous bleeding manifestations.
- Patients with BSS have thrombocytopenia, large platelet size, and a defect in the platelet GPIb-V-IX complex, leading to impaired binding of VWF and adhesion.
- Patients with Glanzmann thrombasthenia have absent or decreased platelet GPIIb-IIIa, leading to impaired binding of fibrinogen and absent aggregation to all of the usual agonists except ristocetin.
- Patients with δ -storage pool deficiency have decreased dense granule contents; some patients may have associated albinism, nystagmus, and neurologic manifestations.
- Patients with the gray platelet syndrome have decreased a-granule contents.
- In a substantial number of patients with abnormal aggregation responses, the underlying mechanisms are unknown. Some patients have defects in platelet activation and signaling mechanisms.

Acquired disorders of platelet function

Alterations in platelet function occur in many acquired disorders of diverse etiology (Table 11-7), of which drugs are the most frequent. Besides the typical antiplatelet drugs, nonsteroidal anti-inflammatory drugs, serotonin reuptake inhibitors (SSRIs) (and other antidepressants), and anticonvulsive drugs are the most widely prescribed. In most of the non-drug-induced acquired platelet function disorders, the specific biochemical and pathophysiological aberrations leading to platelet dysfunction are poorly understood. In some, such as MPNs, there is production of intrinsically abnormal platelets by the bone marrow. In others, the dysfunction results from an interaction of platelets with exogenous factors, such as artificial surfaces (cardiopulmonary bypass), compounds that accumulate in plasma due to impaired renal function, and antibodies; while in liver disease, platelet function is often secondarily

Table 11-7 Disorders in which acquired defects in platelet function are recognized

Uremia
Myeloproliferative neoplasms
Acute leukemias and myelodysplastic syndrome
Dysproteinemias
Cardiopulmonary bypass
Acquired storage pool deficiency
Acquired von Willebrand disease
Antiplatelet antibodies
Drugs and other agents

impaired due to preactivation of platelets by low levels of thrombin. As with inherited disorders of platelet function, in acquired disorders, bleeding is usually mucocutaneous with a wide and unpredictable spectrum of severity. The usual laboratory tests that suggest platelet dysfunction include abnormal results in studies of platelet aggregation or the platelet function analyzer 100 (PFA-100). The bleeding time and the PFA-100 are not reliable discriminators, because these tests may be variably abnormal or normal, even in individuals with impaired platelet aggregation responses. In patients with acquired platelet dysfunction, the correlation between abnormalities in platelet aggregation studies and clinical bleeding remains weak.

Drugs that inhibit platelet function

Many drugs affect platelet function (see video in online edition). Moreover, the impact of concomitant administration of multiple drugs, each with a mild effect on platelet function, is unknown. Because of their widespread use, aspirin and nonsteroidal anti-inflammatory agents are an important cause of platelet inhibition in clinical practice. Aspirin ingestion results in inhibition of platelet aggregation and secretion upon stimulation with ADP, epinephrine, and low concentrations of collagen. Aspirin irreversibly acetylates and inactivates platelet cyclooxygenase (COX-1), leading to the inhibition of synthesis of endoperoxides (prostaglandin G₂ and H₂) and TxA₂. Typically, it is recommended to wait 7 to 10 days after cessation of aspirin ingestion to perform studies intended to assess platelet function and elective invasive procedures to ensure that the antiplatelet effect is gone. Nonsteroidal anti-inflammatory drugs also impair platelet function by inhibiting the cyclooxygenase enzyme. Compared with aspirin, the inhibition of cyclooxygenase by these agents generally is short-lived and reversible (1 to 2 days). Cyclooxygenase-2 inhibitors do not inhibit platelet aggregation responses.

Ticlopidine, clopidogrel, and prasugrel are orally administered thienopyridine derivatives that inhibit platelet function by irreversibly binding to the platelet P2Y12 receptor at the ADP-binding site. In contrast, ticagrelor is a reversible inhibitor of platelet function that binds to P2Y12 at a site different from ADP and allosterically blocks access of ADP to the receptor. These drugs prolong the bleeding time and inhibit platelet aggregation responses to several agonists, including ADP, collagen, epinephrine, and thrombin, to various extents depending on agonist concentrations. The active drug/metabolites of the irreversible antiplatelet drugs (aspirin, clopidogrel, prasugrel) disappear from the circulation within a relatively short time (aspirin 1 hour; clopidogrel ~5 hours; prasugrel ~8 to 10 hours). However, ticagrelor reaches such high plasma levels that it is present in the circulation for 72 to 96 hours despite its relatively short half-life of 7 to 8 hours. This has major implications for patient management. While hemostasis can be normalized after cessation of the irreversible platelet function inhibitors within a reasonable time by platelet transfusion, they are rather ineffective in the case of ticagrelor and any invasive procedures with increased bleeding risk should be postponed by 96 hours, if possible.

GPIIb-IIIa receptor antagonists are compounds that inhibit platelet fibrinogen binding and platelet aggregation. These include the Fab fragment of a monoclonal antibody against the GPIIb-IIIa receptor (abciximab), a synthetic peptide containing the RGD sequence (eptifibatide), and a peptidomimetic (tirofiban). They are potent inhibitors of aggregation in response to all agonists (except ristocetin) and prolong the bleeding time. DITP (secondary to drug-dependent antibodies) occurs in 0.2% to 1.0% of patients on first exposure to GPIIb-IIIa antagonists (see “Drug-induced immune thrombocytopenia” above).

A host of other medications and agents, including oncologic drugs and food substances, inhibit platelet responses, but the clinical significance for many is unclear. β -lactam antibiotics, including penicillins and cephalosporins, inhibit platelet aggregation responses and may contribute to a bleeding diathesis at high doses. The platelet inhibition appears to be dose dependent, taking approximately 2 to 3 days to manifest and 3 to 10 days to abate after drug discontinuation. The clinical significance of the effect of antibiotics on platelet function remains unclear. The general context in which bleeding events are encountered in patients on antibiotics prevents identification of the precise role played by antimicrobials because of the presence of concomitant factors (eg, thrombocytopenia, DIC, infection, vitamin K deficiency). Avoidance or discontinuation of a specifically indicated antibiotic usually is not necessary.

Evidence is growing that SSRIs inhibit platelet function *in vivo*. Serotonin in plasma is taken up by plate-

lets, incorporated into dense granules, and secreted upon platelet activation. SSRIs inhibit the uptake of serotonin, platelet aggregation, and secretion responses to activation. In epidemiologic studies, patients on SSRIs have had increased gastrointestinal bleeding and increased bleeding with surgery.

Given the increasing use of herbal medicines and food supplements, their role and interaction with pharmaceutical drugs need to be considered in the evaluation of patients with unexplained bleeding.

Myeloproliferative neoplasms

Bleeding tendency, thromboembolic complications, and qualitative platelet defects are all recognized in MPNs, which include essential thrombocythemia, polycythemia vera, chronic idiopathic myelofibrosis, and chronic myelogenous leukemia. Platelet function abnormalities result principally from development from an abnormal stem cell clone, but some alterations may be secondary to enhanced platelet activation *in vivo*. The clinical impact of *in vitro* qualitative platelet defects, which are observed even in asymptomatic patients, is unclear.

Numerous studies have examined platelet function and morphology in patients with MPNs. Large platelets may be observed. Under the electron microscope, findings include reduction in dense and α -granules, alterations in the open canalicular and dense-tubular systems, and reduced mitochondria. The bleeding time is prolonged in a minority (17%) of MPN patients and does not correlate with an increased risk of bleeding. Platelet aggregation responses are highly variable in MPN patients and often vary in the same patient over time. Decreased platelet responses are more common, although some patients demonstrate enhanced responses to agonists. In one analysis, responses to ADP, collagen, and epinephrine were decreased in 39%, 37%, and 57% of patients, respectively. The impairment in aggregation to epinephrine is more commonly encountered than with other agonists; however, a diminished response to epinephrine is not pathognomonic of an MPN. Platelet abnormalities described in MPN include decreased platelet α_2 -adrenergic receptors, TxA₂ production, and dense granule secretion and abnormalities in platelet surface expression of GPIIb-IIIa complexes, GPIb, and GPIa-IIa.

Platelets from patients with polycythemia vera and myelofibrosis, but not essential thrombocythemia or chronic myelogenous leukemia, have been shown to have reduced expression of the TPO receptor (Mpl) and reduced TPO-induced tyrosine phosphorylation of proteins. MPN patients have been reported to have defects in platelet-signaling mechanisms. An acquired decrease in plasma VWF has been documented in some MPN patients with

elevated platelet counts and may contribute to the hemostatic defect.

Acute leukemias and myelodysplastic syndromes

The major cause of bleeding in these conditions is thrombocytopenia. In patients with normal or elevated platelet counts, however, bleeding complications may be associated with platelet dysfunction and altered platelet and megakaryocyte morphology. Acquired platelet defects associated with clinical bleeding are more common in acute myelogenous leukemia but also have been reported in acute lymphoblastic and myelomonoblastic leukemias, hairy cell leukemia, and myelodysplastic syndromes.

Dysproteinemias

Excessive clinical bleeding may occur in patients with dysproteinemias, and this appears to be related to multiple mechanisms including platelet dysfunction, specific coagulation factor abnormalities, hyperviscosity, and alterations in blood vessels because of amyloid deposition. Qualitative platelet defects occur in some patients and have been attributed to coating of platelets by the paraprotein. The bleeding tendency may be aggravated by concomitant inhibition of VWF by the paraprotein.

Uremia

Patients with uremia are at an increased risk for bleeding complications. The pathogenesis of the hemostatic defect in uremia remains unclear, but major factors include platelet dysfunction and impaired platelet–vessel wall interactions, comorbid conditions, anemia, and the concomitant use of medications that affect hemostasis. The bleeding time may be prolonged.

Multiple platelet function abnormalities are recognized in uremia, including impaired adhesion, aggregation, and secretion. These hemostatic defects may be linked to the accumulation of dialyzable and nondialyzable molecules in the plasma of patients with renal failure. One such compound, guanidinosuccinic acid, accumulates in plasma, inhibits platelets in vitro, and stimulates generation of nitric oxide, which inhibits platelet responses by increasing levels of cellular cyclic guanosine monophosphate.

Aggressive hemodialysis ameliorates the uremic bleeding diathesis in most patients. Hemodialysis and peritoneal dialysis are equally effective. Platelet transfusion is indicated in the management of acute major bleeds. Other treatments including DDAVP, cryoprecipitate, and conjugated estrogens have also been shown to be beneficial. Elevation of the hematocrit with packed red blood cells or recombinant erythropoietin may improve platelet adhesion and correct mild bleeding in uremic patients. The beneficial effect of red blood cells has been attributed to

rheologic factors whereby the red blood cells exert an outward radial pressure promoting platelet–vessel wall interactions. Other factors predisposing to bleeding in patients with renal failure include concomitant administration of antiplatelet agents or anticoagulant medications.

Acquired SPD

Several patients have been reported in whom dense granule SPD appears to be acquired. This defect probably reflects the release of dense granule contents because of in vivo platelet activation or production of abnormal platelets. Acquired SPD has been observed in patients with antiplatelet antibodies, systemic lupus erythematosus, chronic ITP, DIC, HUS, renal transplant rejection, multiple congenital cavernous hemangiomas, MPN, acute and chronic leukemias, severe valvular disease, and in patients undergoing cardiopulmonary bypass.

Acquired von Willebrand disease (AVWD)

Acquired VWD (AVWD) is an uncommon bleeding disorder. Most patients are older (median age 62 years) and do not have previous manifestations or a family history of a bleeding diathesis. Patients with MPNs and thrombocytosis demonstrate an inverse relationship between plasma VWF levels and platelet count. AVWD has been documented in patients with severe aortic stenosis and congenital valvular heart disease, and in those with left ventricular assist devices due to shear stress–induced loss of the high-molecular-weight multimers of VWF from plasma. It may also result from decreased VWF synthesis or secretion in patients with myxedema. Laboratory findings in AVWD include a prolonged bleeding time and decreased plasma levels of VWF and factor VIII. Most patients exhibit a type 2 VWD pattern with a selective reduction in large VWF multimers and a decreased VWF activity-to-antigen ratio. The goals of treatment in AVWD are to raise plasma VWF levels (using DDAVP or VWF-containing concentrates), to treat or prevent bleeding, and to address the underlying condition. More information on AVWD may be found in Chapter 10.

Antiplatelet antibodies and platelet function

Binding of antibodies to platelets may produce several effects—including accelerated destruction, platelet activation, cell lysis, aggregation, secretion of granule contents, and outward exposure of phosphatidylserine. In ITP, antibodies are directed against specific platelet surface membrane GPs that play a major role in normal platelet function including GPIb, GPIIb-IIIa, GPIa-IIa, GPVI, and glycosphingolipids. Patients with ITP are generally assumed to have normal or supranormal platelet function. However, some may have impaired platelet function due

to antiplatelet antibodies that interfere with platelet function. Other patients may have autoantibodies that impair platelet function but do not induce accelerated platelet clearance or thrombocytopenia.

KEY POINTS

- Alterations in platelet function are described in many acquired disorders of diverse etiologies; the clinical significance remains unclear in many cases.
- A careful drug history should be taken in any patient suspected to have platelet dysfunction.
- Aspirin, nonsteroidal anti-inflammatory agents, and other medications are a major cause of acquired platelet dysfunction.
- Patients with MPNs may have altered platelet function that contributes to bleeding manifestations.
- High platelet counts, as observed in MPN patients, may be associated with a loss of high-molecular-weight multimers of VWF in plasma.
- Patients with renal failure may have impaired platelet function related to accumulation of substances in plasma that inhibit platelet function. Vigorous dialysis is a major part of management of platelet dysfunction in these patients.



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Laboratory hematology

TRACY I. GEORGE AND ANNE M. WINKLER

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Introduction

Hematology laboratory tests are ordered and interpreted within the context of a specific patient; for example, a routine screening or preoperative assessment, or in the setting of an illness for diagnosis or follow-up. Clinical judgment is applied in both the selection of tests and in their interpretation. Some unexpected results may require confirmation, particularly if there is a question about the integrity of the specimen (eg, sample mislabeling, heparin contamination, wrong collection tube or volume of blood, delayed processing). Additional causes of inaccurate laboratory results include analytical and postanalytical errors, although these are less common than preanalytical errors.

Terminology

Sensitivity, specificity, and positive or negative predictive values are defined using the following clinical variables: true positive (TP; assay correctly identifies a disease in those who have it), false positive (FP; assay incorrectly identifies disease when none is present), true negative (TN; assay correctly excludes a disease in those without it), and false negative (FN; assay incorrectly excludes disease when it is present).

Sensitivity [$TP/(TP+FN) \times 100$] is the ability of a test to detect a true abnormality; as the sensitivity of a test increases, the risk of an FP result increases (increasing sensitivity comes at the cost of decreasing specificity). Very sensitive tests are helpful for screening, by ruling out a diagnosis or disease when the test is negative (high negative predictive value).

Specificity [$TN/(TN+FP) \times 100$] is the ability of a test to detect a normal result if the abnormality is not present; as the specificity increases, the risk of an FN result increases. Specific tests are useful for confirmation, by ruling in a diagnosis or disease when the test is positive (high positive predictive value).

Precision is reproducibility of a value during repeated testing of a sample.

Accuracy is the ability of a test to obtain the assigned value of an external standard (run as though it were a clinical sample).

Predictive value is the likelihood that an abnormal test indicates a patient with the clinical abnormality (*positive predictive value* [$TP/(TP+FP) \times 100$], “positive in disease”) or the likelihood that a normal test indicates a patient without the abnormality (*negative predictive value* [$TN/(TN+FN) \times 100$], “negative in health”). Positive



The online version of this chapter contains an educational multimedia component on von Willebrand disease.

Conflict-of-interest disclosure:

Dr. George: consultancy: Roche.
Dr. Winkler: employment: Instrumentation Laboratory.

Off-label drug use: Dr. George: not applicable. Dr. Winkler: not applicable.

and negative predictive values depend on the frequency of the abnormality being sought in the population, as well as the sensitivity and specificity of the laboratory method.

Reference ranges are derived from a sample of a well population and typically reflect the results of 95% (mean ± 2 standard deviations) of disease-free individuals. The reference ranges of some assays are determined by the results of 99% of disease-free individuals.

The *receiver operating characteristic curve* plots the sensitivity (true positive rate) on the y-axis versus the FP rate (100-specificity) on the x-axis for different cut-points of a diagnostic test. This allows one to see the trade-off between sensitivity and specificity for a specific laboratory test, where an increase in sensitivity is accompanied by a decrease in specificity. The closer the curve to the top left corner of the graph, the more accurate the test.

Specific laboratory tests

Automated blood cell counting

In addition to complete blood counts (CBC) and the traditional 5-part leukocyte differential counts, newer hematolgy analyzers can also provide quantitative and qualitative information about reticulocytes and reticulocyte-specific indices, nucleated red blood cells (NRBC), immature granulocytes, and platelet parameters such as platelet immaturity. Because of the large number of cells counted from each blood sample and analysis using multiple physical principles and sophisticated software, hematolgy analyzers produce accurate and precise CBCs and leukocyte differential counts, with the exception of basophils, because of their low frequency. Many laboratories no longer report band neutrophils, because accurate and precise identification by automated and morphologic techniques is poor and their clinical significance (if any) appears minimal, with the possible exception of neonatal sepsis and febrile children with sickle cell disease. Hematolgy analyzers provide excellent sensitivity to distinguish between normal and abnormal samples via operator alerts (flags) prompting microscopic review of a stained peripheral blood smear for selected samples. As a result, a variable percentage of hospitalized patients' samples require review of a stained blood smear.

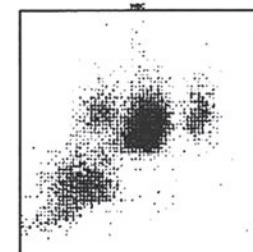
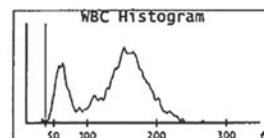
Automated blood cell counters use various technologies to enumerate and classify blood cells (Figure 12-1). Most platforms available for clinical use utilize at least 2 of the following techniques.

Aperture impedance (Coulter principle)

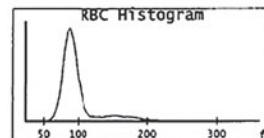
Cells diluted in a conducting solution are counted, and their volume is determined by measuring change in elec-

WBC	4.8	$10^3/\mu\text{L}$
NE %	64.5	%
LY %	20.7	%
MO %	7.3	%
EO %	6.4	%
BA %	1.1	%

NE #	3.1	$10^3/\mu\text{L}$
LY #	1.0	$10^3/\mu\text{L}$
MO #	0.4	$10^3/\mu\text{L}$
EO #	0.3	$10^3/\mu\text{L}$
BA #	0.1	$10^3/\mu\text{L}$



RBC	4.15	$10^6/\mu\text{L}$
HGB	13.1	g/dL
HCT	36.9	%
MCV	88.8	fL
MCH	31.6	pg
MCHC	35.6	g/dL
RDW	12.2	%



PLT	173	$10^3/\mu\text{L}$
MPV	9.0	fL

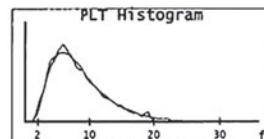


Figure 12-1 Data and histograms performed on a Beckman-Coulter LH 750 automated hematology analyzer from a healthy adult. The WBC, RBC, and platelet (PLT) histograms represent cell volumes determined by impedance. The second histogram from the top displays WBC light scatter in a flow cell; the y-axis indicates forward scatter and volume, and the x-axis indicates side scatter due to granularity and nuclear features. Basophils (BA) are detected by an alternative physical property not displayed. EO, eosinophil; HCT, hematocrit; LY, lymphocyte; MO, monocyte; NE, neutrophil.

trical resistance as they flow through a narrow aperture and interrupt a direct electrical current. Software analysis defines red blood cells (RBC), white blood cells (WBC), and platelets based on volume limits, and calculates RBC and platelet indices.

Optical absorbance

This technique exploits the cytochemical reaction of an intracellular enzyme, such as myeloperoxidase, to absorb white light from a tungsten light source after the addition of a substrate. Light absorbance is proportionate to the intensity of the enzyme-catalyzed reaction. This technique may be used to detect and distinguish peroxidase-containing cell types (neutrophils, eosinophils, monocytes) from peroxidase-negative lymphocytes and basophils.

Optical light scatter

This method monitors the light-scattering properties of blood cells, using a technique similar to that employed by flow cytometers. Cells pass in single file across the path of a unifocal laser. The amount of light scattered at a low angle from the incident light path is proportional to cell volume. The amount of light scattered at a wide angle depends on such factors as cytoplasmic granules and nuclear shape. All of the major hematology analyzers use light-scattering technology.

Fluorescence

In addition to the physical properties of cells, fluorochrome-labeled antibodies recognizing cell surface or intracellular epitopes and fluorescent dyes further refine the separation of individual cell types. A variety of reagents can be used to distinguish platelets (thiazole orange, anti-CD41, anti-CD42b, anti-CD61), reticulocytes (thiazole orange, anti-CD4K, RNA dyes), fetal RBCs (antihemoglobin F/D), NRBCs (propidium iodide, Draq 5, other DNA-binding dyes), neutrophils, lymphocytes, and blasts.

Erythrocyte analysis

Automated blood cell counters measure the number (RBC count, reported in units of $10^{12}/L$) and volume (mean corpuscular volume [MCV], reported in units of fL or $10^{-15} L$) of RBCs, and hemoglobin concentration (reported in units of g/dL) after lysing RBCs; all other parameters are calculated. Hemoglobin is converted by potassium ferricyanide to cyanmethemoglobin, and absorbance is measured by a spectrophotometer at 540 nm. Some analyzers use a cyanide-free method. RBCs may be spuriously increased in patients with hyperleukocytosis and giant platelets, and decreased in the presence of RBC agglutinins, cryoglobulins, and *in vitro* hemolysis. Hemoglobin measurements can be elevated artifactually by increased sample turbidity because of leukocytosis, paraproteinemia, carboxyhemoglobinemia, hyperbilirubinemia, or hyperlipidemia. Sulfhemoglobin also interferes with hemoglobin values.

MCV is calculated from the distribution of individual RBC volumes. This measurement can be elevated artifactually by agglutination of RBCs, resulting in measurement of more than 1 cell at a time; hyperglycemia, causing osmotic swelling of the RBC; and spherocytes, which have decreased deformity.

Automated hematocrit (%) is calculated by multiplying the MCV by the RBC number: hematocrit = $MCV \times RBC \text{ count} \times 10^6 \times 100$. Some analyzers directly measure hematocrit.

The mean corpuscular hemoglobin (MCH) is expressed in picograms ($10^{-12} g$). The MCH is calculated by dividing

hemoglobin (g/L) by RBC count ($10^{12}/L$). An elevated MCH can be an artifact of increased plasma turbidity.

The MCH concentration (MCHC) is expressed in grams of hemoglobin per deciliter of packed RBCs. The MCHC is calculated by dividing the hemoglobin concentration (g/dL) by the hematocrit (%) $\times 100$. Any artifact affecting the hematocrit or hemoglobin determinations can alter the MCHC; for example, spherocytosis and agglutination.

The RBC distribution width (RDW) is the coefficient of variation of RBC size (anisocytosis): standard deviation/MCV. The RDW is used in the evaluation of anemia. The RDW is more frequently elevated with microcytic anemias due to iron deficiency anemia than to thalassemia or anemia of chronic disease; it is also elevated more frequently with macrocytic anemias due to vitamin B₁₂ or folate deficiency compared to liver disease, hypothyroidism, or reticulocytosis. Myelodysplastic syndromes with ring sideroblasts, or RBC transfusions in patients with severe microcytic or macrocytic anemias, can produce a dimorphic RBC pattern with a very wide RDW.

Reticulocyte counts

Automated hematology analyzers use dyes or fluorescent techniques to detect residual mRNA in young erythrocytes, and all provide accurate reticulocyte counts expressed as a percentage of RBCs or as an absolute number. Some blood cell counters provide reticulocyte indices that are analogous to the standard RBC indices, including reticulocyte hemoglobin content (CHr) on Advia analyzers (Siemens, Tarrytown, NY) and reticulocyte MCV (MCVr) on several other analyzers. Reductions in CHr and MCVr reflect inadequate hemoglobin synthesis in real time, providing immediate information about functional iron deficiency when other biochemical markers of iron availability may be difficult to interpret due to inflammatory conditions. CHr is particularly useful for assessing response to erythropoiesis-stimulating agents and iron therapy in renal dialysis patients. The immature reticulocyte fraction is another parameter that measures immature reticulocytes and serves as a marker of erythropoiesis in the bone marrow, where very low values reflect bone marrow aplasia and high values reflect increased erythropoiesis in the bone marrow.

Red blood cell fragments

The reliable identification of RBC fragments (schistocytes) is important in the diagnosis of microangiopathies such as hemolytic uremic syndrome, thrombotic thrombocytopenic purpura (TTP), transplant-associated thrombotic microangiopathy, and disseminated intravascular coagulation

(DIC). The Sysmex XE and Siemens Advia systems both take advantage of the small size of RBC fragments to distinguish them from normal RBCs. Although both platforms are noted to overestimate the number of RBC fragments in a specimen, this parameter can be used by the laboratory for identification of specimens needing microscopic examination.

Nucleated red blood cells

Circulating NRBCs occur in newborns; however, beyond this period, the presence of NRBCs is abnormal and associated with various hematopoietic stresses, including hemolytic anemias, myeloproliferative neoplasms, metastatic cancer to the bone marrow, and hypoxia. All major hematology analyzers enumerate NRBCs and correct WBC and lymphocyte counts for interference from NRBC analysis.

Leukocyte analysis

To differentiate lymphocytes, monocytes, neutrophils, eosinophils, and basophils, most instruments use impedance and/or light scattering, plus additional physical properties. Beckman Coulter and Sysmex analyzers utilize radiofrequency conductivity, and Advia analyzers use peroxidase staining. Leukocyte differentials typically are reported as percentages of WBC and as absolute counts. Automated blood cell counters provide sensitive flags and warnings for immature granulocytes and monocytes and abnormal lymphocytes. Instrument manufacturers continue to refine technologies to report extended differentials to quantify neutrophil precursors, typically as immature granulocytes. Some Sysmex analyzers identify a subset of WBCs called hematopoietic progenitor cells.

Platelet analysis

Automated blood cell counters enumerate platelets, measure volume, and calculate mean platelet volume (MPV). Associations between MPV and acquired mechanisms of thrombocytopenia suggest that MPV increases with peripheral destruction of platelets because of increased megakaryocyte ploidy and production of larger platelets, whereas MPV decreases when platelet production is suppressed. Platelets undergo time-dependent shape changes when exposed to ethylenediaminetetraacetic acid (EDTA), and may lead to inaccurate MPV results and thus diminished clinical utility in laboratories where blood samples are not tested for prolonged periods of time. Inaccurate automated platelet counts can result from fragmented RBCs, congenital (inherited macrothrombocytopenia disorders such as May-Hegglin anomaly) or acquired (myeloproliferative neoplasms or idiopathic thrombocytopenic pur-

pura) macrothrombocytes, and EDTA-mediated platelet clumping because of immunoglobulin M (IgM) autoantibodies. Hematology analyzers provide sensitive warnings for abnormal platelet populations requiring manual smear review to confirm or revise platelet counts. Analogous to reticulocytes, young platelets (also called reticulated platelets) contain detectable mRNA. Currently, only certain analyzers provide an immature platelet fraction based on the analysis of cell volume and fluorescence intensity of mRNA binding dye. Potential applications include differentiating thrombocytopenia due to megakaryopoiesis failure from peripheral destruction and determining earlier evidence of marrow regeneration following stem cell transplantation or response to a thrombopoietin mimetic drug.

Examination of peripheral blood smears

Blood smears are air-dried and typically stained with either Wright or May-Grünwald-Giemsa stains and can be prepared by automated slide maker/stainers, which can be interfaced with hematology analyzers. Some analyzers (eg, the Roche Cobas m511) “print” blood onto a glass slide and stain with proprietary dyes and then derive all CBC measurements from the slide, as well as generate cell images onto a computer screen. Microscopic examination or image analysis of stained blood smears can identify morphologic abnormalities that automated hematology analyzers nonspecifically flag or, rarely, miss. Microscopic examination begins at low power ($\times 10$), scanning for platelet clumps or abnormal, large nucleated cells that may be located along the lateral and leading edges of the smear. At higher magnification ($\times 50$ and $\times 100$), the optimal area to examine RBC, platelet, and leukocyte morphologies and to perform WBC differentials is the transitional area between the thick part of the smear and the leading edge (Table 12-1), where there are only a few overlapping RBCs and central pallor of normal RBCs is evident. Hematologists should review a patient’s peripheral smear as part of any consultation potentially involving qualitative or quantitative blood cell abnormalities.

The accuracy of manual WBC differentials suffers from small sample size (typically 100 cells), distributional bias of WBCs on the smear, and variable interobserver agreement regarding cell classification. Advances in digital microscopy and image analysis can improve the accuracy of WBC classification while reducing technical time. For example, the CellVision DM96 (CellaVision, Lund, Sweden) scans a stained blood smear, makes digital images of WBCs, classifies them, and presents the sorted WBC images to an operator to confirm or reclassify. When compared with manual differentials, au-

Table 12-1 Red blood cell abnormalities*

Abnormality	Description	Cause	Disease association
Acanthocytes (spur cells)	Irregularly spiculated red cell	Altered membrane lipids	Liver disease, abetalipoproteinemia, postsplenectomy
Basophilic stippling	Coarse punctate basophilic inclusions	Precipitated ribosomes	Lead toxicity, thalassemias, pyrimidine-5'-nucleotidase deficiency
Bite cells (degmacytes)	Smooth semicircle taken from 1 edge	Heinz body pitting by spleen	G6PD deficiency, drug-induced oxidant hemolysis
Burr cells (echinocytes)	Short, evenly spaced spicules	May be related to abnormal membrane lipids	Usually artifactual; also uremia
Cabot ring	Circular, blue, threadlike inclusion with dots	Nuclear remnant	Postsplenectomy, hemolytic anemia, megaloblastic anemia
Howell-Jolly bodies	Small, discrete basophilic dense inclusions; single	Nuclear remnant	Postsplenectomy, hemolytic anemia (acute), megaloblastic anemia
Pappenheimer bodies	Small dense basophilic granules of varying size; multiple	Iron-containing siderosomes or mitochondrial remnant	Sideroblastic anemia, iron overload
Schistocytes (helmet cells)	Distorted, fragmented cell, with 2–3 pointed ends, loss of central pallor	Mechanical distortion in the microvasculature by fibrin strands; damage by mechanical heart valves	Microangiopathic hemolytic anemia, prosthetic heart valves, severe burns
Spherocytes	Spherical cell with dense appearance and absent central pallor; usually decreased diameter	Decreased membrane redundancy	Hereditary spherocytosis, autoimmune hemolytic anemia
Stomatocytes	Mouth- or cuplike deformity	Membrane defect with abnormal cation permeability	Hereditary stomatocytosis, artifact
Target cell (codocyte)	Target-like appearance, often hypochromic	Increased redundancy of cell membrane	Liver disease, postsplenectomy, thalassemia, HbC
Teardrop cell (dacrocyte)	Distorted, drop-shaped cell		Myelofibrosis, myelophthisic anemia

Modified from Kjeldsgaard C, ed. Practical Diagnosis of Hematologic Disorders. 5th ed. Chicago, IL: ASCP Press; 2010.

*Blood smear abnormalities can be artifacts of poor slide preparation or viewing the wrong part of the smear.

When pediatric marrow specimens are examined, it is understood that erythroid hyperplasia is present at birth because of high levels of erythropoietin. Lymphocytes may comprise 40% of the marrow cellularity in children <4 years of age, and eosinophils are present in higher numbers than in adults.

Perls or Prussian blue reactions are used to identify hemosiderin in NRBCs (sideroblastic iron) and histiocytes (reticuloendothelial iron). See Table 12-2 for other cytochemical stains.

Ring sideroblasts are abnormal NRBCs with at least 5 blue-staining iron granules surrounding at least one-third of the nucleus. These iron granules are present in mitochondria surrounding the nuclear membrane. Iron staining of the biopsy can underestimate the marrow iron stores because of the loss of iron during decalcification.

Stomatocytes must be confirmed on examination of fresh blood under the microscope (wet preparation), as these are a common artifact in air-dried blood smears.

Hb C, hemoglobin C.

tomated morphologic differentials demonstrate excellent routine differential accuracy and sensitivity to detect blasts. In addition, stored images can be reviewed at remote locations, such as outpatient clinics. However, it should be noted that this image analysis is performed at higher magnification, focusing on individual cells of interest.

Supravital stains are used to detect RBC inclusions; these are manual methods and labor intensive. Crystal violet detects denatured hemoglobin inclusions (Heinz bodies) because of enzymopathies such as glucose-6-

phosphate dehydrogenase (G6PD) deficiency; brilliant cresyl blue is used to precipitate and detect unstable hemoglobins (hemoglobin H in α -thalassemias).

The bone marrow aspirate and biopsy

The most frequent indications for bone marrow biopsy include: unexplained cytopenias; unexplained leukocytosis, erythrocytosis, or thrombocytosis; staging of lymphoma and some solid tumors (particularly in patients with cytopenias or other findings suggestive of bone marrow involvement);

diagnosis of plasma cell neoplasms (myeloma and monoclonal gammopathy of uncertain significance); evaluation of systemic iron levels; evaluation of an infectious process; and unexplained splenomegaly. Bone marrow aspirate and biopsy are commonly performed by collecting specimens from the posterior iliac crest. Bone marrow aspirates also can be obtained from the sternum. In newborns and young infants, marrow aspirates often are obtained from the anterior tibia. Quality smears require adequate spicule harvesting because perispicular areas are the most representative areas to examine.

The bone marrow aspirate and touch preparations from trephine samples are air-dried and usually stained with either Wright or May-Grünwald-Giemsa stain. The aspirate smear is used for cytologic examination of the bone marrow cells and for performing the differential. Bone marrow core biopsies are most commonly fixed in formalin, and the biopsy specimen is decalcified and embedded in paraffin; 3- to 4- μm sections are then cut and stained with hematoxylin and eosin or Giemsa stain. Bone marrow aspirates can also be sent for microbiologic culture to work up suspected infections.

Immunohistochemical stains

A large array of specific antibodies detected by enzymatic formation of a colored product linked to the antigen-antibody complex are now available for use on bone marrow biopsies or other tissues. Many cytochemical stains

(Table 12-2), such as tartrate-resistant acid phosphatase (TRAP) and myeloperoxidase, have been converted into immunohistochemical reactions.

Immunohistochemistry (IHC) is used on marrow biopsies and clot sections as an alternative or adjunct to flow cytometry. The advantage of IHC is the ability to correlate morphology with phenotype. IHC can be used to phenotype undifferentiated tumors, lymphoproliferative disorders, and atypical lymphoid infiltrates. In patients whose marrow cannot be aspirated (dry tap), IHC can be performed on the biopsy section. IHC also can be used on sections of lymph nodes or other tissues when there is concern about lymphoma or some other neoplastic disease.

Preparation of bone marrow samples for ancillary studies

Bone marrow collected in EDTA is adequate for both flow cytometry and molecular analysis. Bone marrow collected for cytogenetic studies should be collected in heparin.

Paraffin-embedded tissue can be used for polymerase chain reaction (PCR) of genomic DNA sequences, depending on the laboratory. Reverse transcriptase PCR assays require that RNA preparations be performed as early as possible to prevent digestion by ubiquitous nucleases.

Flow cytometry

The most common applications of flow cytometry in hematology include the detection of cell surface or cyto-

Table 12-2 Cytochemical stains

Cytochemical stain	Substrate and staining cells
Myeloperoxidase	Primary granules of neutrophils and secondary granules of eosinophils. Monocytic lysosomal granules stain faintly.
Sudan black B	Stains intracellular phospholipids and other lipids. Pattern of staining is similar to myeloperoxidase.
Naphthol AS-D chloroacetate esterase (specific esterase)	Granulocytes stain; monocytes do not stain. Can be used in biopsies to stain granulocytes and mast cells.
α -Naphthyl butyrate (nonspecific esterase)	Stains monocytes, macrophages, and histiocytes. Does not stain neutrophils.
α -Naphthyl acetate (nonspecific esterase)	Megakaryocytes stain with α -naphthyl acetate but not α -naphthyl butyrate
Terminal deoxynucleotidyl transferase (TdT)	Intranuclear enzyme. Stains thymocytes and lymphoblasts. Some myeloblasts stain positively.
Tartrate-resistant acid phosphatase (TRAP)	Stains an acid phosphatase isoenzyme. Positive staining in hairy cell leukemia, Gaucher cells, activated T lymphocytes.
Periodic acid-Schiff (PAS)	Detects intracellular glycogen and neutral mucosubstances. Positive staining in acute lymphoblastic leukemia, acute myeloid leukemia, erythroleukemia, and Gaucher cells.
Toluidine blue	Detects acid mucopolysaccharides. Positive in mast cells and basophils.

Table 12-3 Specimen allocation for ancillary studies

Clinical problem	Ancillary techniques
Pancytopenia	Flow cytometry (LGL, hairy cell leukemia, PNH clone, AML) Cytogenetics (AML, MDS) Molecular genetics
Acute myeloid leukemia	Flow cytometry (phenotyping, minimal residual disease) Cytogenetics and FISH Molecular genetics, including NGS
Lymphoproliferative disorder	Flow cytometry (phenotyping, prognostic markers, minimal residual disease in B-ALL) Cytogenetics: t(1;19) in B-ALL, t(14;18) in follicular lymphomas, etc. FISH (<i>MYC</i> , <i>BCL2</i> , <i>BCL6</i>) Molecular genetics (clonality, etc.) Immunohistochemistry (phenotyping, prognostic markers)
Myeloproliferative neoplasms	Cytogenetics FISH (<i>BCR-ABL1</i>) Molecular genetics (<i>BCR-ABL1</i> , <i>JAK2</i> , <i>CALR</i> , <i>MPL</i>)
Plasma cell disorders	Flow cytometry (phenotyping, labeling index, minimal residual disease) Cytogenetics FISH

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; LGL, large granular lymphocyte leukemia; MDS, myelodysplastic syndrome; NGS, next generation sequencing; PNH, paroxysmal nocturnal hemoglobinuria.

plasmic proteins using fluorescent-labeled monoclonal antibodies and the assessment of DNA content using DNA-binding dyes.

Flow cytometry is used for phenotyping populations of cells, enumerating early progenitors for stem cell transplantation, detecting minimal residual disease, detecting targets for immunotherapy, and assessing the presence of prognostic markers. See Table 12-3 for a summary of clinical uses of flow cytometry and ancillary studies.

Gating is necessary to identify cells of interest in a mixed population of cells. Three major leukocyte populations (lymphocytes, monocytes, and neutrophils) can be defined using light scatter. Forward-angle scatter (FS; low angle) measures cell size, and side-light scatter (SS) measures internal cellular granularity. Lymphocytes have the lowest FS and SS signals, monocytes have intermediate FS and SS signals, and neutrophils have high SS and slightly lower FS signals. Blasts generally have low FS and SS.

The most common method for gating different cell populations is by plotting right-angle SS against CD45. Cells can be separated based on the intensity of staining they display with the conjugated antibody that is classified as either bright or dim. Lymphocytes are bright CD45 and have a low SS signal, neutrophils are dim to moderately bright CD45 and have a high SS signal, and monocytes are bright CD45 and have an intermediate SS. Blasts have low SS and dim to negative CD45 expression, the latter being more common in blasts of lymphoid lineage.

Flow cytometry also can be used to detect populations of natural killer (NK) cells. NK cells express CD2, CD7, CD16, and CD56 and show variable expression of CD57 and CD8. NK cells do not express CD3, and the absence of CD3 expression can be used to differentiate NK cells from T cells.

In addition to determining cell lineage, flow cytometry can be used to detect prognostic markers. For example, flow cytometric analysis of the tyrosine kinase ZAP-70 can be used to subdivide chronic lymphocytic leukemia (CLL) into prognostic groups. Positivity for ZAP-70 is highly correlated with unmutated immunoglobulin heavy chain variable region (IgV_h), a feature of CLL arising from pregerminal center cells, and patients with pregerminal center CLL have a decreased overall survival when compared with patients with CLL arising from postgerminal center cells. Positivity for CD38 by flow cytometric analysis also is correlated with unmutated IgV_h , but the correlation is not as strong as it is with ZAP-70. In addition, expression of CD49d, an integrin alpha subunit, by CLL cells is associated with a more aggressive disease course.

Uncommitted hematopoietic progenitors are CD34+ and CD38-; expression of CD38 is evidence of lineage commitment. Myeloid maturation is characterized by the following maturational sequence: colony-forming units—erythroid granulocyte, macrophage, and megakaryocyte (CFU-GEMM, CD34+, MHC class II+, CD33-/+); and followed by colony-forming units—granulocyte and macrophage (CFU-GM, CD34+, major histocompatibility

complex [MHC] class II⁺, CD33⁺, CD13^{-/+}, CD15^{-/+}). Neutrophil precursors then progressively lose MHC class II and CD33 and gain CD11b, CD16, and CD32. Monocytes retain expression of MHC class II and CD33 and also gain expression of CD14 and CD64.

Appearance of CD71, loss of CD34 and CD33, and decreased expression of CD45 characterize erythroid maturation. With further differentiation, CD71 expression decreases, glycophorin expression increases, and CD45 disappears.

Megakaryocytic differentiation is indicated by the expression of glycoprotein (GP) IIb (CD41). GPIIb/IIIa (CD61) expression increases as CD34 expression decreases. GPIb (CD42b) is expressed at the promegakaryocyte stage. GPV (CD42d) expression increases with megakaryocyte differentiation. Differential expression of CD41, CD42b, and CD61 can also be used to study platelets and diagnose platelet disorders, including Glanzmann thrombasthenia and Bernard-Soulier syndrome.

B- and T-cell precursors express terminal deoxynucleotidyl transferase (TdT), human leukocyte antigen (HLA)-DR, and CD34. B-cell differentiation is indicated by the expression of CD19 followed by CD10. As B cells mature, they lose cell surface expression of CD34 and CD10 and express IgM on the cell surface. Expression of surface IgM is associated with the expression of mature B-lymphocyte markers (CD20, CD21, CD22, and CD79b). Mature B cells express an immunoglobulin heavy chain, such as IgM, and either the κ- or a λ-light chain. A predominant expression of 1 type of light chain on the cell surface of a population of B cells is known as light-chain restriction and is indicative of a monoclonal process.

T-cell precursors express TdT, HLA-DR, and CD34. Differentiation is indicated by the expression of cytoplasmic CD3 and CD7, followed by the expression of CD2 and CD5. The common thymocyte also expresses CD1, CD4, and CD8. The mature helper or inducer lymphocyte expresses CD2, CD3, CD4, and CD5 and may express CD7. The mature suppressor or cytotoxic T lymphocyte expresses CD2, CD3, CD4, CD5, and CD8 and may express CD7. T-cell neoplasms may be associated with abnormal expression patterns of T-cell antigens, and the abnormal pattern may be detected by flow cytometric analysis. See Tables 12-4 through 12-10 for useful CD markers.

Flow cytometry can be used to diagnose paroxysmal nocturnal hemoglobinuria (PNH). PNH is associated with the absence of glycosylphosphatidylinositol (GPI)-anchored membrane proteins, including 2 complement regulatory molecules: decay accelerating factor (DAF, CD55) and protectin (MIRL, CD59). The absence of these proteins from the cell surface of erythrocytes can be detected by flow cytometry using antibodies to CD55 and CD59, respec-

tively. Alternatively, PNH granulocytes are detected by the absence of GPI anchor binding by FLAER, an Alexa 488 labeled variant of aerolysin. Flow cytometry technology can discriminate between fetal and adult RBCs or Rh⁺ and Rh⁻ RBCs during pregnancy and postpartum and can identify RBC skeletal disorders, such as hereditary spherocytosis.

Cytogenetics

Cytogenetic analysis can be performed from cultured (indirect) and uncultured (direct) preparations. In the indirect assay, cells are grown so that mitotic forms can be visualized and distinct chromosomal banding patterns can be assessed (conventional cytogenetics). Growing or culturing the cells increases the mitotic rate and improves chromosome morphology. Mitogens may be useful in improving the yield of karyotyping abnormal cells and are particularly useful when analyzing mature B- or T-cell processes. A cytogenetic clone is defined by a minimum of 2 mitotic cells with the same abnormality. Constitutional chromosome abnormalities, associated with either congenital genetic syndromes or normal variants, are determined on peripheral blood T lymphocytes grown in culture with phytohemagglutinin, a T-cell mitogen.

Fluorescence in situ hybridization (FISH) is a cytogenetic technique that uses specific fluorescent-labeled DNA probes to identify each chromosomal segment. FISH can be performed using either cultured or uncultured preparations. In the uncultured technique, the assay is performed using nuclear DNA from interphase cells that are affixed to a microscope slide. FISH can be performed using bone marrow or peripheral blood smears or fixed and sectioned tissues; decalcification typically interferes with FISH assays.

Hybridization of centromere-specific probes is used to detect monosomy, trisomy, and other aneuploidies. Chromosome-specific libraries, which paint the chromosomes, are useful in identifying marker chromosomes or structural rearrangements, such as translocations. Translocations and deletions also can be identified in interphase or metaphase by using genomic probes that are derived from the breakpoints of recurring translocations or within the deleted segment. Multiplex FISH (spectral karyotyping) consists of simultaneously painting all chromosomes in a cell using different colors for each chromosome.

Cytogenetics is particularly useful in the subclassification of acute myeloid leukemias and in confirming the diagnosis and prognosis of B-cell neoplasias. CLL, acute leukemias, B-cell lymphomas, and multiple myeloma all have cytogenetic abnormalities that can be detected using either conventional cytogenetics or FISH. While the sensitivity of FISH is higher at approximately 10^{-4} compared with

Table 12-4 Clinically useful cluster-of-differentiation (CD) markers

Marker	Lineage association
Progenitor cells	
CD34	Progenitor cells, endothelium
CD38	Myeloid progenitors, T, B, NK cells, plasma cells, monocytes, CLL subset
B-cell markers	
CD10	Pre-B-lymphocytes, germinal center cells, neutrophils
CD19	B cells (not plasma cells or follicular dendritic cells)
CD20	B cells (not plasma cells)
CD21	Mature B cells, follicular dendritic cells, subset of thymocytes
CD22	Mature B cells, mantle zone cells (not germinal center cells)
CD23	B cells, CLL
CD79b	B cells (not typical CLL)
CD103	Intraepithelial lymphocytes, hairy cell leukemia, T cells in enteropathic T-cell lymphoma
FMC7	B cells (not typical CLL), hairy cell leukemia
T-cell markers	
CD2	Pro- and pre-T cells, T cells, thymocytes, NK cells, some lymphocytes in CLL and B-ALL
CD3	Thymocytes, mature T cells, cytoplasm of immature T cells
CD5	Thymocytes, T cells, B cells in CLL, B cells in mantle cell lymphoma
CD4	Helper T cells, monocytes, dendritic cells, activated eosinophils, thymocytes
CD7	Pro- and pre-T cells, T cells, thymocytes, NK cells, some myeloblasts
CD8	Suppressor T cells, NK cells, thymocytes
CD25	Activated T and B cells, adult T-cell leukemia/lymphoma
NK/cytotoxic T-cell markers	
CD16	NK cells, monocytes, macrophages, neutrophils
CD56	NK cells, myeloma cells
CD57	NK cells, T-cell subset
Myeloid and monocytic markers	
CD13	Monocytes, neutrophils, eosinophils, and basophils
CD14	Monocytes, macrophages, subset of granulocytes
CD33	Myeloid lineage cells and monocytes
CD117	Immature myeloid cells, AML, mast cells
Monocytes	
CD11c	Monocytes, macrophages, granulocytes, activated B and T cells, NK cells, hairy cell leukemia
CD15	Myeloid lineage cells and monocytes
CD64	Monocytes, immature myeloid cells, activated neutrophils
Megakaryocytic markers	
CD41	Platelets and megakaryocytes (GPIIb)
CD42	Platelets and megakaryocytes (CD42a: GPI; CD42b: GPIb)
CD61	Platelets, megakaryocytes, endothelial cells (GPIIb/IIIa)
Erythroid markers	
CD71	Transferrin receptor is upregulated upon cell activation
CD235a	Glycophorin A

AML, acute myeloid leukemia; B-ALL, B-lineage acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia.

Table 12-5 Acute myeloid leukemia phenotyping using the FAB classification

	HLA-DR	CD34	CD33	CD13	CD11c	CD14	CD41	CD235a
M0	+	+	+	+/-	+/-	-	-	-
M1	+	+	+	+	+/-	+/-	-	-
M2	+/-	+/-	+	+	+/-	+/-	-	-
M3	-	-	+	+	+/-	-	-	-
M4	+	+/-	+	+	+	+	-	-
M5	+	-	+	+	+	+	-	-
M6	+/-	-	-	-	+/-	-	-	+
M7	+/-	+/-	+/-	-	-	-	+	+

FAB, French-American-British.

Table 12-6 B-lineage acute lymphoblastic leukemia phenotyping

	TdT	CD19	CD10	CD20	Cyto-μ	Surface Ig
Pro-B	+	+	-	-	-	-
Pre-pre-B (common ALL)	+	+	+	-	-	-
Pre-B	+	+	+	+/-	+	-
Early B (Burkitt)	-	+	+	+	-	+

Cyto-μ, cytoplasmic mu; Ig, immunoglobulin; Tdt, terminal deoxynucleotidyl transferase.

Table 12-7 T-lineage acute lymphoblastic leukemia phenotyping

Surface	TdT	CD7	CD2	CD5	CD1a	sCD3	cCD3	CD4/CD8
Prothymocyte	+	+	+	-	-	-	+	-/-
Immature thymocyte	+	+	+	+	-	-	+	-/-
Common thymocyte	+	+	+	+	+	+/-	+	+/-
Mature thymocyte	-	+	+	+	-	+	+	CD4 or CD8+
Mature T cell	-	+	+	+	-	+	+	CD4 or CD8+

cCD3, cytoplasmic CD3; sCD3, surface CD3; Tdt, terminal deoxynucleotidyl transferase.

a sensitivity of 10^{-2} for conventional cytogenetics, FISH requires the use of location-specific probes to identify specific aneuploidies or translocations, whereas conventional cytogenetics detects all chromosomal abnormalities if cells show mitotic activity. Rapid FISH assays may have turnaround times of only a few hours, while most standard FISH assays require 1 to 2 days. Conventional cytogenetics requires cells to grow and thus the turnaround times vary from 4 up to 10 days.

Molecular diagnostics

PCR is designed to permit selective amplification of a specific target DNA sequence within total genomic DNA or a complex complementary DNA population. Partial DNA sequence information from the target sequences is required. Two oligonucleotide primers, which are specific

for the target sequence, are used. The primers are added to denatured single-stranded DNA. A heat-stable DNA polymerase and the 4 deoxynucleotide triphosphates are used to initiate the synthesis of new DNA strands. The newly synthesized DNA strands are used as templates for further cycles of amplification. The amplified DNA sequence can be detected by electrophoresis on an agarose gel, and visualization can be accomplished by the use of a DNA dye; alternatively, the amplified DNA can be sequenced directly in an automatic sequencer.

Uses of PCR in clinical laboratories include detection of the break cluster region-Abelson tyrosine kinase (*BCR-ABL1*) translocation in chronic myeloid leukemia and detection of select genes such as the Janus kinase-2 (*JAK2*) mutation in polycythemia vera, essential thrombocythemia, and primary myelofibrosis. PCR is appropriate

Table 12-8 Common B-cell neoplasms

	CD20	CD5	CD10	CD23	CD43	cIg	sIg	Cyclin D1	Other
CLL/SLL	+	++	-	++	++	5%+	+	-	CD200+, CD79b+
LPL	++	-	-	-	+/-	+	+	-	
PLL	++	+/-		-			++	-	
HCL	++	-	-	-	-	-	+	+/-	CD11c+, CD25+, CD103+
MCL	++	++	-	-	++	-	++	++	CD200-
MZL	++	-	-	-	+/-	+/-	++	-	
FL	++	-	60%+	-/+	-	-	++	-	BCL2+
LCL	++	10%+	25%-50%+	-	+/-	+/-	+/-	-	BCL2+ in 30%-40%
BL	++	-	+	-	-	+	+	-	BCL2-
Myeloma	-/+	-	Occ +	-	+	++	-	15%-20%+	CD56+, CD38+, CD138+

BL, Burkitt lymphoma; cIg, cytoplasmic immunoglobulin; CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; HCL, hairy cell leukemia; LCL, large-cell lymphoma; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; Occ, occasionally; MZL, marginal zone lymphoma; PLL, B-cell prolymphocytic leukemia; sIg, surface immunoglobulin; SLL, small lymphocytic lymphoma.

Table 12-9 Common mature T-cell and NK-cell neoplasms

	sCD3	cCD3	CD5	CD7	CD4	CD8	CD30	CD16	CD56	EBV
T-PLL	+dim	+	+	+	+/-	-/+	-	-	+	-
T-LGL	+	+	-	+	-	+	-	+	-	-
NK leukemia	-	-	-	+/-	-	+/-	-	-	+	+
EN-NK/T	-	+	-	+/-	-	-	-	+	+	+
HSTL	+	+	-	+	-	-	-	+	+/-	-
Ent-T lym	+	+	+	+	-	+/-	+/-	-	-	-
SCPTL	+	+	+	+	-	+	+/-	-	-	-
PTCL-NOS	+	-	+/-	+/-	+/-	+/-	+/-	-	+	+/-
AILT	+	+	+	+	+/-	-	-	-	+	+/-
ALCL	+	-	+/-	+/-	+/-	+/-	+	-	-	-

AILT, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; cCD3, cytoplasmic CD3; sCD3, surface CD3; EBV, Epstein-Barr virus; Ent-T lym, enteropathy-associated T-cell lymphoma; EN-NK/T, extranodal natural killer/T-cell lymphoma; HSTL, hepatosplenic T-cell lymphoma; NK leukemia, natural killer cell leukemia; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; SCPTL, subcutaneous panniculitis-like T-cell lymphoma; T-LGL, T-cell large granular lymphocyte leukemia; T-PLL, T-prolymphocytic leukemia.

for selected situations including the rapid diagnosis of select mutations important in acute myeloid leukemia, such as the detection of promyelocytic leukemia-retinoic acid receptor alpha (*PML-RARA*) in acute promyelocytic leukemia and mutations in isocitrate dehydrogenase 1 and 2 (*IDH1/2*). In addition to rapid turnaround time, PCR is also appropriate for detection of mutations in genes like FMS-like tyrosine kinase 3 (*FLT-3*) locus and calreticulin (*CALR*), which contain large indels which may be missed by current mapping algorithms in massive parallel sequencing assays. Finally, PCR is appropriated for standardized minimal residual disease testing, for example, *BCR-ABL1* in chronic myeloid leukemia.

DNA sequencing is important in the identification of point mutations. The earlier Sanger (chain termination) technique has been eclipsed by next-generation sequencing (NGS) technology (massive parallel sequencing), which has a high throughput capacity and thus makes parallel analysis of many genes possible. The clinical uses—including diagnosis, predictors of response to therapy, and risk stratification—are employed in a variety of hematologic malignancies, including myeloma, leukemias, and lymphoma, as well as identifying hereditary genetic mutations that predispose patients to inherited hematologic disorders. The tradeoff of the analysis of mutations in many genes is time, with most NGS panels requiring sophisticated

Table 12-10 Immunohistochemical diagnosis of Hodgkin lymphoma

	CD45	CD30	CD15	CD20	CD3	PAX5
CHL (RS cells)	—	+	+	—	—	Dim+
NLPHL (LP cells)	+	—	—	+	—	+
B-cell lymphoma	+	+/-	—	+	—	+
T-cell lymphoma	+	+/-	+/-	—	+	—

CHL, classic Hodgkin lymphoma; NLPHL, nodular lymphocyte-predominant Hodgkin lymphoma; LP, lymphocyte predominant; RS, Reed-Sternberg.

bioinformatics pathways and curating of variants detected that require 1 to 2 weeks for results. In the U.S., there are published recommendations for describing the significance of variants (mutations) detected with Tier 1 through Tier 4 grading. Sanger sequencing is still used for select genes and to confirm NGS results in some genes (eg, *CEBPA*).

Miscellaneous laboratory hematology methods

Erythrocyte sedimentation rate

The erythrocyte sedimentation rate (ESR) measures a physical phenomenon—the opposing forces of gravity and buoyancy on RBCs when blood is suspended in an upright tube—and is expressed in millimeters per hour. Elevated plasma proteins, primarily fibrinogen and immunoglobulins, neutralize the RBC membrane negative charge, facilitating rouleaux formation and more rapid sedimentation because of increased mass per surface area. The clinical utility of ESR generally is poor except for selected rheumatologic disorders, and it is not an appropriate screening test in asymptomatic patients. Conditions associated with elevated ESR include malignancies, infections, and inflammatory conditions (particularly polymyalgia rheumatic and temporal arteritis), as well as hematologic conditions such as cold agglutinin disease, cryoglobulinemia, and plasma cell dyscrasias-related M proteins. ESR reference ranges increase with age and are higher for women. Additional variables affect ESR: anemia and macrocytosis can cause faster sedimentation, whereas sickle cells by impeding rouleaux formation, and microcytosis cause slower sedimentation. The modified Westergren method (EDTA blood diluted 4:1 in sodium citrate and put in a 200 mm vertical glass tube) is the preferred manual method. Automated ESR analyzers monitor sedimentation for shorter periods, extrapolate to millimeters per hour, and correlate reasonably well with the Westergren method.

Solubility testing for hemoglobin S

Manual qualitative methods to detect hemoglobin S (Hb S) rely on visual detection of turbidity when blood containing Hb S is added to a solution containing a reduc-

ing agent, detergent to lyse red blood cells, and high-concentration salt buffer. Deoxygenated Hb S forms tactoids that defract and reflect light; whereas nonsickling hemoglobins remain soluble, allowing the detection of lines or letters when viewed through the hemolysis solution. A positive solubility test cannot discriminate between Hb S trait, homozygous Hb S, Hb S/β-thalassemia, or other combinations that include Hb S. FP results can occur due to paraprotein or cryoglobulin precipitation, and FN results can occur in anemic (hemoglobin <7.0 g/dL) sickle trait individuals or when the Hb S concentration is <2.6 g/dL. Because the concentration of Hb S in affected newborns is low, sickle solubility testing should not be performed on infants <6 months old because of the risk of FN results. If used as a screening test, a positive solubility test requires evaluation by an alternative method to confirm and quantify Hb S and to identify coexisting nonsickling hemoglobinopathies or thalassemias. Other rare hemoglobinopathies produce a positive solubility test, including Hb C Harlem, and if coinherited with Hb S, they produce a sickle cell disease phenotype.

Hemoglobin electrophoresis

Methods to separate normal (Hb A, A₂, and F) and abnormal hemoglobins, primarily based on differences in charge, include alkaline and acid gel electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC), and capillary electrophoresis (Figure 12-2). No method can definitively identify and quantify all hemoglobin variants, and any abnormal hemoglobin identified by the method chosen for screening must be confirmed by an alternative method (including solubility test for presumed Hb S). HPLC and capillary electrophoresis analyzers are fully automated, provide precise measurements of normal and variant hemoglobins, and are well suited for laboratories performing many analyses to diagnosis hemoglobins S, C, and E, as well as other uncommon hemoglobinopathies and β-thalassemia trait (elevated Hb A₂, microcytic anemia). For optimal genetic counseling, DNA analysis may be appropriate to completely characterize α-thalassemias and some uncommon thalassemias and hemoglobinopathies.

G6PD testing

Evaluation for inherited RBC enzymopathies is appropriate in patients with nonspherocytic, nonimmune-mediated hemolytic anemia. X-linked inheritance of G6PD deficiency is the most common RBC enzyme defect and is associated with hemolysis during oxidative stresses because of acute illness, medications, or (rarely) ingestion of fava beans. Decreased G6PD activity diminishes nicotinamide adenine dinucleotide phosphate (NADPH) production

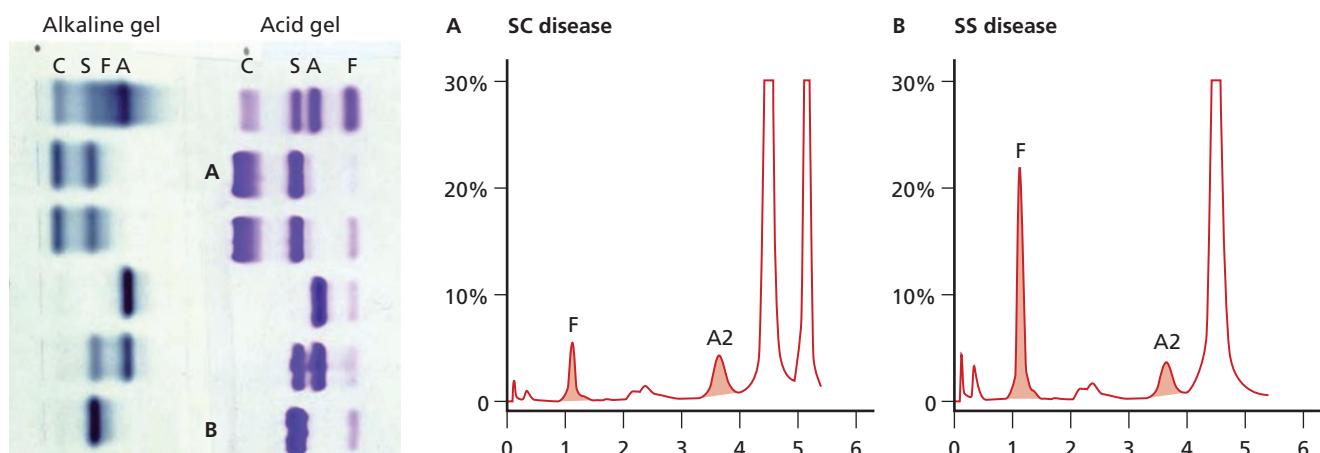


Figure 12-2 Examples of alkaline and acid gel electrophoresis and high-performance liquid chromatography patterns for a patient with hemoglobin SC disease (A) and a patient with homozygous sickle cell disease (Hb SS) (B).

and prevents reduction of methemoglobin by reduced glutathione, leading to denatured hemoglobin (Heinz bodies) and shortened RBC survival. Sensitive qualitative screening tests for G6PD deficiency include dye decolorization and fluorescent spot tests, which monitor NADPH-dependent chemical reactions. FN results may occur if testing is performed during or shortly after a hemolytic event in individuals (typically African and African American males) with the A-mutation because enzyme activity is near normal in reticulocytes. Pyruvate kinase deficiency is the second most common RBC enzyme defect, presenting with chronic hemolysis of variable severity and an autosomal recessive inheritance pattern. In patients with hemolysis, a suspicion for an RBC enzymopathy, and normal G6PD screening, blood should be sent to a reference laboratory that offers quantitative testing for G6PD activity and a panel of additional RBC enzyme tests.

Hereditary red cell skeletal disorders

The unique flexibility of a RBC depends on its lipid bilayer attachment to an underlying scaffold of α - and β -spectrin dimers via transmembrane proteins and other linking molecules. Inherited quantitative and qualitative RBC cytoskeleton defects are an infrequent cause of non-immune chronic hemolysis, but these defects are relatively more common in people of northern European ancestry. The most common phenotype is hereditary spherocytosis (HS), with an estimated incidence of 1 in 2,000 whites (see Chapter 8 for more structural details). The intensity of hemolysis can vary from severe anemia to a completely compensated state. In about 75% of HS cases, there is an autosomal-dominant inheritance pattern, and diagnosis can be made on the basis of family history, a negative

direct antiglobulin test, anemia with reticulocytosis, mild splenomegaly, and spherocytes on blood smear. In suspected cases of HS that appear to be sporadic, or if data on family members are unavailable, laboratory studies are indicated to confirm loss of the RBC membrane, anchoring proteins, or spectrin. Although spherocytes are more susceptible to lysis when suspended in hypotonic saline solutions because of a decreased surface area or volume, increased osmotic fragility (OF) is an insensitive screening test for mild and compensated HS, and OF can produce FP results. A more sensitive and specific method is detection of decreased eosin-5-maleimide (EMA) binding by flow cytometry due to loss of RBC membrane proteins. Hereditary elliptocytosis causes minimal, if any, anemia and is a morphologic diagnosis (normal OF and EMA binding). Hereditary pyropoikilocytosis is caused by inheritance of both qualitative and quantitative RBC skeletal defects, which produce severe hemolytic anemia, deranged red cell morphologies, and decreased EMA.

Hemostasis testing

Hemostasis involves multiple molecular and cellular interactions to initiate and regulate platelet aggregation (primary hemostasis) and coagulation (secondary hemostasis) at the site of vascular injury to produce a durable “patch” without occluding blood flow. Laboratory evaluation of hemostasis is performed in several clinical settings, including screening of asymptomatic patients before selective invasive procedures and patients with underlying disorders associated with bleeding complications, evaluation of patients with personal or family histories of abnormal bleeding or bruising, assessment of inherited and

acquired thrombotic risk factors, and anticoagulant drug monitoring.

Hemostasis screening typically consists of a prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet count. Abnormal screening tests require additional clinical and laboratory investigation to determine the underlying etiology. Mucosal bleeding, menorrhagia, petechiae, and ecchymoses suggest primary hemostatic disorders such as von Willebrand disease (VWD) and qualitative platelet disorders, whereas hematomas, hemarthroses, and delayed bleeding suggest a coagulation factor defect.

Testing for thrombophilia is usually performed when a patient has a venous thromboembolic event (VTE) in the absence of compelling acquired risk factors, such as major surgery or trauma, cancer and its treatment, and immobility. The decision to test for a predisposition to VTE also depends on the patient's gender, age, history of thrombosis, family history of thrombosis, and whether the results would influence duration of anticoagulant therapy. Laboratory testing for inherited deficiencies of coagulation regulatory proteins should be done after a patient has completed treatment for a VTE and is in stable health. Levels of protein C (PC), protein S (PS), and antithrombin (AT) can decrease during the acute phase of a VTE, and can also be reduced during anticoagulation treatment; PC and PS levels are reduced by warfarin and AT levels are decreased during unfractionated heparin (UFH) therapy. In addition, the direct oral anticoagulants (DOAC), dabigatran, rivaroxaban, apixaban, edoxaban, and betrixaban, can also interfere with thrombophilia testing. Lupus anticoagulant (LAC) testing should ideally be performed before anticoagulation is initiated, in conjunction with serologic assays (anticardiolipin [aCL] and anti- β -2-glycoprotein I IgM and IgG antibodies), and abnormal results should be repeated at least 12 weeks later to determine whether they are persistently abnormal to fulfill the laboratory classification criteria for antiphospholipid syndrome (APS). Genetic thrombophilia testing (factor V Leiden [FVL] and prothrombin 20210 gene mutations) can be ordered at any time and are unaffected by clinical status or medications. Heparin-induced thrombocytopenia (HIT) and TTP are unique acquired thrombocytopenia disorders with the potential for thrombotic complications. Laboratory test results can provide subsequent support for these diagnoses, but immediate therapeutic interventions should be based on clinical assessment in the absence of a rapid test.

Two major forms of anticoagulant therapy—warfarin antagonism of vitamin K-dependent γ -carboxylation of coagulation factors II, VII, IX, and X, proteins C and S; and UFH—require therapeutic drug monitoring because of unpredictable anticoagulant activities. Efforts to har-

monize interlaboratory monitoring of warfarin with the PT ratio and heparin with the aPTT have led to the international normalized ratio (INR) and heparin activity (chromogenic anti-Xa) assays, respectively. The DOACs (dabigatran, rivaroxaban, apixaban, edoxaban and betrixaban) do not require therapeutic drug monitoring; however, assays based upon anti-IIa or anti-Xa are available in some laboratories to measure drug concentrations in special clinical situations such as bleeding, breakthrough thrombosis, suspected noncompliance, populations at risk for drug accumulation, and prior to urgent surgery or administration of thrombolytic therapy.

The following sections provide specific information regarding hemostasis laboratory methods as they apply to the aforementioned clinical situations.

Preanalytical variables

Most laboratory errors occur in the preanalytical phase, which includes specimen collection, collection container composition and anticoagulant, tube fill volume and mixing, sample transport and processing, and duration and temperature of routine and frozen specimen storage. Samples sent for coagulation testing are especially susceptible to preanalytical variables.

For coagulation testing, the proportion of whole blood to sodium citrate anticoagulant volume is 9:1. Filling a tube with less than the recommended volume or collecting blood in the same proportions from a polycythemic patient increases the concentration of citrate in the plasma compartment, leading to incomplete recalcification when a fixed volume of CaCl_2 is added, and results in artifactual prolongation of the PT or aPTT.

Hemolysis, icterus, and lipemia/turbidity (HIL) in patient samples can also interfere with accurate measurement of coagulation assays. HIL can be attributable to *in vitro* processes, resulting from incorrect sampling procedures, transport, or storage of specimens, causing hemolyzed samples; *in vivo* RBC lysis (eg, from hereditary or acquired conditions such as autoimmune hemolytic anemia, thrombotic microangiopathy, DIC), causing hemolysis; physicochemical mechanisms such as the formation of chylomicrons and very-low-density lipoprotein after a high fat meal, administration of intravenous lipids or an underlying metabolic disorder such as diabetes, acute pancreatitis, or steroid administration, causing sample lipemia/turbidity; and the presence of free (unconjugated) and direct (conjugated) bilirubins in icteric samples. Of the preanalytical errors in the coagulation laboratory, spurious hemolysis is the leading cause (19% to 40%) while icterus and lipemia are less common. HIL increases the spectrometric absorbance of the plasma sample and leads to high background ab-

sorbance readings, which may interfere with analyzers that use light-scattering clot detection methods, thereby compromising clot detection and accuracy of test results. Though analyzers may not be affected by or compensate for HIL, the quality of these specimens should be questioned because HIL can cause activation of coagulation.

In addition, exogenous interferences such as presence of an anticoagulant or coagulation factor replacement therapy may also interfere with plasma-based coagulation testing. Heparin contamination due to blood collection from central lines can cause a prolonged aPTT. A prolonged aPTT that corrects when repeated after treatment of plasma with a heparin-neutralizing agent confirms heparin contamination. Alternatively, a prolonged thrombin time (TT) and a normal reptilase time, which utilizes a snake venom not neutralized by heparin-accelerated AT, confirms the presence of heparin. Most PT reagents contain heparin-neutralizing agents such as Polybrene, making this screening test insensitive to heparin contamination. Many coagulation tests performed on plasma from patients taking oral direct factor-IIa (dabigatran) and

factor Xa (rivaroxaban, apixaban, edoxaban, betrixaban) anticoagulants are at risk for either positive or negative biases, which can be clinically important (Table 12-11). Current strategies to extend the half-life of FVIII and FIX concentrates include fusion to the Fc domain of human immunoglobulin, PEGylation, and albumin fusion, which can cause interference in one-stage clotting assays depending upon the reagent used.

If a patient sample has an interference, the patient's test result may either be rejected or reported. If reported, the laboratory should annotate the result with a comment to indicate the presence and effect of the interference on the patient's result.

Screening coagulation testing and associated abnormalities

Most *in vivo* coagulation reactions are believed to be initiated by tissue factor activation of factor VII. Important interactions occur between the extrinsic and intrinsic pathways in physiologic *in vivo* hemostasis. Although the division into 2 separate pathways, as shown in Figure 12-3, does not reflect

Table 12-11 Coagulation tests interference caused by direct oral anticoagulants

Test	Factor IIa inhibitor (eg, dabigatran)	Factor Xa inhibitors (eg, rivaroxaban, apixaban)	Comments
aPCr ratio, aPTT based	+ bias	+ bias	Risk of missing FVL
Antithrombin, anti-Xa method	unaffected	+ bias	Risk of missing AT deficiency
Antithrombin, anti-IIa method	+ bias	unaffected	Risk of missing AT deficiency
Factors X, VII, V, II (PT based)	- bias	- bias	Possible inhibitor pattern
Factors PK, HMWK, XII, XI, IX, VIII (aPTT based)	- bias	- bias	Possible inhibitor pattern
LAC testing	abnormal	abnormal	Possible to misclassify as LAC
Protein C clotting assay	+ bias	+ bias	Risk of missing PC deficiency
Protein S clotting assay	+ bias	+ bias	Risk of missing PS deficiency
PT and aPTT	prolonged	prolonged*	
PT 1:1 mix	prolonged	prolonged	Inhibitor pattern
aPTT 1:1 mix	prolonged	prolonged	Inhibitor pattern
Thrombin time	prolonged	unaffected	
Fibrinogen activity (Clauss)	- bias with some methods [†]	unaffected	
Chromogenic anti-Xa monitoring of heparin/LMWH	unaffected	+ bias	Not a quantitative test for rivaroxaban, apixaban, edoxaban, or betrixaban unless calibrated with the specific drug

*Direct Xa inhibitors may have variable effects depending on the drug and drug concentration. In addition, different reagents show different sensitivities.

[†]Effect is method and drug dependent. Most fibrinogen assays show no effect with dabigatran. Bivalirudin can mildly decrease fibrinogen, while argatroban can significantly falsely reduce fibrinogen.

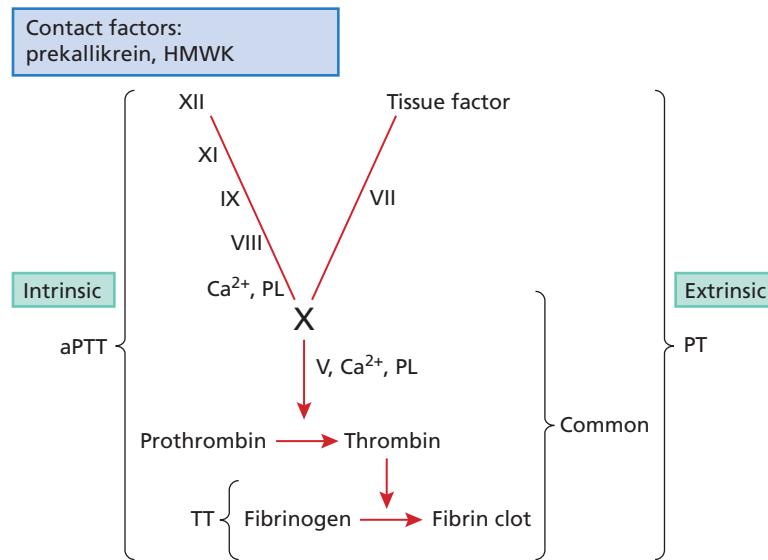


Figure 12-3 Simplified coagulation cascade indicating the intrinsic pathway measured by aPTT, the extrinsic pathway measured by PT, the common pathway (factor X, factor V, prothrombin, and fibrinogen) measured by PT and aPTT, and the conversion of fibrinogen to fibrin measured by TT.

the complex interactions between coagulation factors *in vivo*, it does provide a useful way to interpret screening coagulation test results when evaluating for potential abnormalities of hemostasis.

Prothrombin time

The PT measures the time to form a fibrin clot after adding thromboplastin (source of tissue factor combined with phospholipid) and CaCl₂ to citrated plasma and assesses 3 of the 4 vitamin K-dependent factors (factors II, VII, and X), plus factor V and fibrinogen. Commercial thromboplastins contain either recombinant human tissue factor combined with phospholipid or thromboplastins derived from rabbit or bovine tissues. Almost all PT reagents contain a heparin-neutralizing additive to allow for monitoring of warfarin during concurrent heparin therapy.

Isolated prolongation of the PT most often reflects a deficiency of vitamin K-dependent factors resulting from poor nutrition, inadequate absorption of vitamin K, antagonism of γ -carboxylation of the vitamin K-dependent factors by warfarin, or decreased hepatic synthesis. Causes of an isolated prolonged PT include preanalytical variables, congenital factor deficiencies, acquired inhibitors, and anticoagulants (Figure 12-4).

Congenital deficiencies of factors X, V, II and fibrinogen are rare (1 in 1 million to 2 million people), whereas the estimated prevalence of homozygous factor VII defi-

ciency is 1 in 300,000 people. Some factor VII mutations produce greater PT prolongations with rabbit or bovine tissue factor than with human tissue factor. Therefore, it is important to confirm a suspected congenital factor VII deficiency by measuring factor VII activity with recombinant human thromboplastin. Dysfibrinogenemia occasionally causes a prolongation of the PT without a prolongation of the aPTT, and factor VII inhibitory auto-antibodies are extremely rare.

Warfarin causes a prolonged PT and variably, prolonged aPTT, depending on the degree of factor IX, X, and II deficiencies. Therapeutic monitoring of warfarin depends on the PT. Thromboplastins, however, have different sensitivities to the effects of warfarin. To account for this variability, and to obtain an international sensitivity index (ISI), reagent manufacturers compare PTs obtained with commercial thromboplastin lots to a World Health Organization reference thromboplastin, with the behavior of recombinant or human tissue factor, performed on plasma samples from healthy controls and stable, anticoagulated patients. A sensitive thromboplastin with an ISI of 1.0 is equivalent to human tissue, whereas a thromboplastin with an ISI of 2.0 is relatively insensitive to depletion of vitamin K-dependent clotting factors. The INR is the ratio of the patient's PT to the laboratory's PT geomean raised to the exponent of the thromboplastin ISI. The INR is designed to accurately monitor patients who have been

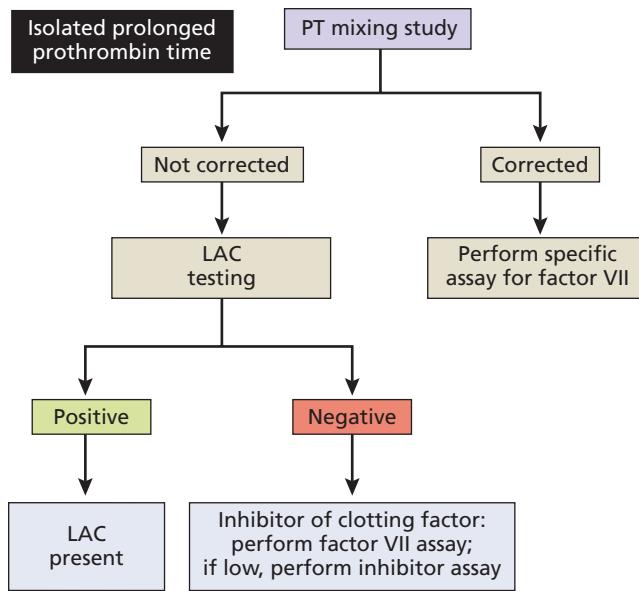


Figure 12-4 Algorithm for evaluation of an isolated prolonged PT.

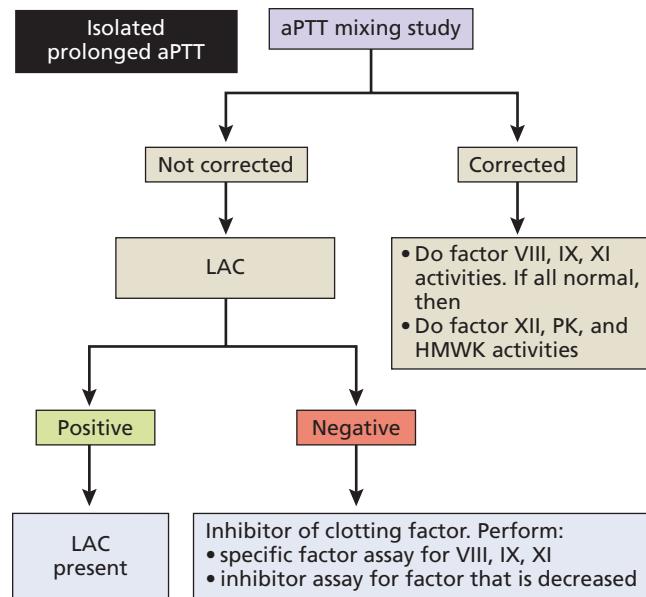


Figure 12-5 Algorithm for evaluation of an isolated prolonged aPTT.

stabilized on warfarin. It is *not* intended for assessing coagulopathies due to liver disease or DOACs because the ISI has not been validated for these conditions.

Activated partial thromboplastin time

The aPTT is a 2-step assay to measure the time to form a fibrin clot after incubation of citrated plasma with phospholipid and negatively charged particles followed by the addition of CaCl_2 . The negative surface and phospholipid activate the contact factors (factor XII, prekallikrein [PK], and high-molecular-weight kininogen [HMWK]) and factor XI. The addition of CaCl_2 permits activation of factor IX and the remaining reactions to proceed to form a fibrin clot.

Causes of an isolated prolonged aPTT include preanalytical variables, congenital factor deficiencies, acquired inhibitors, and anticoagulants (Figure 12-5).

Deficiencies of factors VIII, IX, XI, XII, PK, and HMWK prolong the aPTT. Severe deficiencies of factor XII, PK, and HMWK are rare, typically produce aPTTs >100 seconds and do not cause a bleeding disorder. Depending on the coagulation reagents and analyzer used, for an isolated intrinsic factor deficiency to prolong the aPTT, factor activity is usually 30% to 40%.

Factors VIII and IX deficiencies, or hemophilia A and B, respectively, are X-linked inherited disorders that often are diagnosed early in life due to spontaneous bleeding or a positive maternal family history of hemophilia. Occasionally, diagnosis is delayed until adulthood if it is a mild deficiency (5% to 40%).

Factor XI deficiency should be investigated when a prolonged aPTT is encountered in a person of Ashkenazi Jewish ancestry. Bleeding risk is variable and does not correlate particularly well with the severity of factor XI deficiency.

Patients with type 1 VWD may have a slightly prolonged aPTT if the factor VIII level is low, as von Willebrand factor (VWF) serves to stabilize FVIII. Patients with the type 2 Normandy variant of VWD can have a moderate factor VIII deficiency, while patients with type 3 VWD typically have a severe deficiency of FVIII.

LACs can cause a prolonged aPTT (see additional discussion in assays for thrombophilia). If a prolonged aPTT does not substantially shorten when repeated on a 1:1 mix with pooled normal plasma (PNP), LAC testing or specific factor activities should be performed, depending on the clinical context.

Most hospitals use aPTT-based nomograms to guide UFH anticoagulation. A therapeutic aPTT range is typically determined by collecting plasma samples from patients on heparin and comparing aPTTs to heparin activity using the chromogenic anti-Xa assay. The aPTT therapeutic range in seconds corresponds to an anti-Xa range of 0.3 to 0.7 IU/mL. The aPTT is also used to monitor the parenteral direct thrombin inhibitor argatroban, and the therapeutic target recommended by the manufacturer is 1.5 to 3.0 times the baseline aPTT. Therapeutic infusions of direct thrombin inhibitors also prolong the PT/INR, and the intensity depends on the specific direct

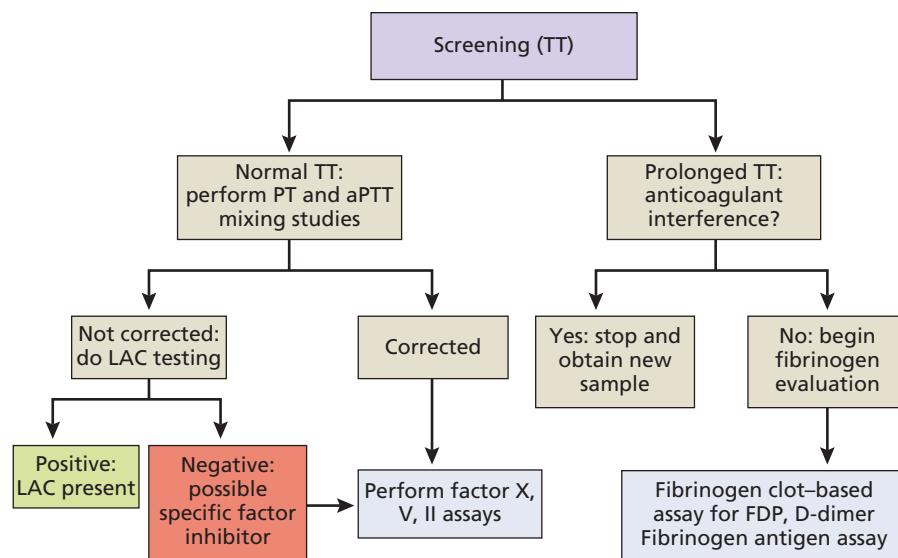


Figure 12-6 Algorithm for evaluation of a prolonged PT and aPTT. FDP, fibrin degradation product; LAC, lupus anticoagulant; TT, thrombin time.

thrombin inhibitor and the thromboplastin reagent. The DOACs can prolong the aPTT and/or PT (Table 12-11); however, these assays cannot be used to predict plasma concentrations. As a result, assays based upon anti-IIa or anti-Xa are available in some laboratories to measure drug concentrations in special clinical situations such as bleeding, breakthrough thrombosis, suspected noncompliance, populations at risk for drug accumulation, and prior to urgent surgery or administration of thrombolytic therapy.

Combined abnormalities of PT and aPTT

Deficiency or inhibition of a factor in the common pathway (factors X, V, II, and fibrinogen), acquired dysfibrinogenemia, DIC, and an LAC can cause combined prolongation of the PT and aPTT (Figure 12-6). Advanced liver disease can cause decreased hepatic synthesis of all coagulation factors, except for factor VIII, and acquired dysfibrinogenemias are suggested by a low fibrinogen level in a functional assay combined with a normal or high level of immunologic fibrinogen (see the section “Fibrinogen assays” in this chapter).

Inhibitors to factor V can develop following exposure to bovine thrombin, which also contains bovine factor V, when combined with fibrinogen to produce “fibrin glue” during surgical procedures to control bleeding. Bovine factor V antibodies may cross-react with human factor V to cause bleeding in some patients. Low factor V activity and specific *in vitro* inhibition of factor V confirm the diagnosis. Fortunately, fibrin glue therapeutics containing either plasma-derived or recombinant human thrombin are now available.

Acquired prothrombin deficiency rarely accompanies LACs, causes moderately prolonged PTs, and can cause abnormal bleeding. The autoantibodies do not produce an inhibitor pattern in mixing studies because they are not directed against the active site of the molecule. Rather, they form immune complexes, increasing the clearance rate and lowering prothrombin activity.

PT and aPTT mixing studies

The purpose of a mixing study is to determine whether a prolonged aPTT or, occasionally, a prolonged PT is more likely due to a deficiency of 1 or more coagulation factors or to an inhibitor. The first step is to exclude contamination with heparin or other anticoagulant by performing a TT, heparin neutralization, or anti-Xa assay. Next, the aPTT or PT is repeated on a 1:1 mixture of patient plasma and PNP, which should provide at least 50% activity for all coagulation factors and substantial correction if a factor deficiency is the cause of a prolonged clotting time. Because factor VIII inhibitors and some LACs manifest their effect in prolonging the aPTT in a time- and temperature-dependent manner, 1:1 mixtures are incubated at 37°C for 1 to 2 hours followed by repeating the aPTT. There is no consensus approach for interpretation of mixing study results, and inflexible requirements such as correction to within the laboratory’s PT or aPTT reference ranges to rule out inhibitor activity can be misleading. One must consider the clinical context and the initial extent of PT and/or aPTT prolongation when assessing the 1:1 mix results. Sometimes mixing studies are not definitive, espe-

cially when an aPTT is mildly prolonged and corrects with mixing, in which case performing both selected factor activity assays and LAC screening may be necessary.

Coagulation factor activity assays

Determination of a specific coagulation factor activity in a patient's plasma typically is performed by one-stage clotting assays performed on automated coagulation analyzers and requires 2 reagents: PNP and plasma deficient in the factor of interest. Combining equal volumes of plasma from a large number of healthy adults averages the interindividual variability for coagulation factors, which typically ranges from 50% to 150%, to produce PNP with 100% activity for all factors. Mixing PNP and factor-deficient plasma in different ratios produces calibrators of known factor activities, which is automated on most analyzers. PTs are performed for factors VII, X, V, and II, and aPTTs are performed for the intrinsic pathway factors. When the factor activities of the calibrators are plotted against the corresponding PT or aPTT results on logarithmic axes, a line or curve is generated. Then, a PT or aPTT is performed on patient plasma mixed with factor-deficient plasma, and the corresponding activity is determined from the calibration curve.

Additionally, factor levels are determined at a minimum of 3 serial dilutions of a patient's plasma, and the results, corrected for the dilution factor, are compared. If an inhibitor is present, the factor activity appears to increase with dilution and results are nonparallel to the calibration curve. To determine whether the inhibitor interference is specific for a factor, such as factor VIII, or nonspecific like an LAC, may require performance of additional testing.

Current strategies to extend the half-life of FVIII and FIX concentrates include fusion of recombinant FVIII or FIX to the Fc domain of human immunoglobulin, PE-Gylation, and albumin fusion. These modifications have shown an improvement in half-life; however, they have also been shown to accentuate discrepancies with one-stage clotting assays as compared to chromogenic assays. These differences are reagent specific and laboratories should be aware of the sensitivity of their reagents to these modified products.

Factor VIII and IX chromogenic activity assays exist but are not widely used, with the exception of specialty laboratories. The end point of these assays is cleavage of a small peptide by an activated coagulation factor that generates a change in color (optical density) proportional to the activity of the factor. Chromogenic assays are more precise and demonstrate lower interlaboratory variability than one-stage clotting assays. However, discrepant one-stage clotting and chromogenic assay results exist, especially in

patients with specific hemophilia A phenotypes or genetic mutations, and when some factor concentrates are assayed.

Inhibitor assays

Inhibitors to factor VIII are detected in 25% to 30% of males with severe hemophilia A due to the development of alloantibodies to infusions of foreign factor VIII. Alloantibody formation to factor IX in males with severe hemophilia B occurs less often. Acquired hemophilia caused by autoantibodies to factor VIII is the most common acquired specific factor inhibitor. A factor VIII antibody is suspected in patients without a significant bleeding history who present with severe bleeding symptoms and coagulation testing shows a prolonged aPTT that fails to fully correct immediately after 1:1 mixing and subsequently prolongs after a 1- to 2-hour incubation of the 1:1 mixture at 37°C. A very low or undetectable factor VIII activity and mild inhibitor patterns for factors IX and XI due to partial inhibition of factor VIII in these aPTT-based activity assays confirm the presence of a specific factor VIII inhibitor. The Bethesda assay determines the potency of a factor VIII inhibitor by incubating dilutions of patient plasma prepared with imidazole buffer combined 1:1 with PNP at 37°C for 2 hours, followed by determination of residual factor VIII activity. The antibody titer is expressed in Bethesda units (BU) equal to the reciprocal of the patient plasma dilution required to obtain recovery of 50% of the expected factor VIII activity in the incubated 1:1 mixture. By definition, 1 BU is defined as the amount of inhibitor producing a residual factor VIII activity of 50%. A titer of 0.5 to 5.0 BU/mL is a low titer and may be overwhelmed with larger infusions of factor VIII, whereas a titer of >10 BU/mL requires treatment of bleeding episodes with a factor VIII bypassing agent, such as recombinant factor VIIa or activated prothrombin complex concentrate or a newly introduced monoclonal antibody, emicizumab. In comparison to the Bethesda assay, the Nijmegen modification or Nijmegen Bethesda assay incorporates buffered PNP and use of FVIII-deficient plasma instead of buffer for dilution and in the control. By reducing the nonspecific degradation of factor VIII during the 2-hour incubation period, the Nijmegen Bethesda assay has improved specificity compared to the Bethesda assay for low-titer inhibitors. Moreover, addition of a heat treatment step (56°C for 30 minutes) to eliminate infused or endogenous FVIII from the sample prior to testing permits accurate testing in recently treated patients.

Fibrinogen assays

The Clauss method is a modified TT in which fibrinogen rather than thrombin is limiting. The time to clot

formation is inversely proportional to fibrinogen activity calibrated against a standard of known concentration and expressed as milligrams per deciliter. The thrombin concentration usually is high enough to not be affected by therapeutic concentrations of heparin but can be affected by direct thrombin inhibitors. Fibrinogen also can be measured in immunologic tests (radial immunodiffusion) to evaluate for possible dysfibrinogenemia.

Thrombin time

The TT measures the time required to convert fibrinogen to a fibrin clot, bypassing the intrinsic, extrinsic, and common pathways. Achieving a normal TT requires sufficient amounts of normal fibrinogen and absence of thrombin inhibitors or substances that impede fibrin polymerization. The reagent is bovine or human thrombin, and the test sample is undiluted citrated plasma.

UFH, low-molecular-weight heparin (LMWH), argatroban, bivalirudin, and dabigatran inhibit thrombin and prolong the TT. Dysfibrinogenemias usually prolong the TT and are suspected if the functional test (fibrinogen activity) is disproportionately low compared with an immunologic measurement of fibrinogen (fibrinogen antigen). Hypofibrinogenemia usually prolongs the TT when levels of fibrinogen are below approximately 90 mg/dL. L-asparaginase can cause hypofibrinogenemia by inhibiting synthesis. Fibrin degradation products in very high concentrations and M proteins can inhibit fibrin polymerization and prolong the TT. Heparin-like anticoagulants (heparan sulfates) have occurred in patients with multiple myeloma and other tumors; they prolong the TT by interacting with AT in a manner similar to heparin, but the reptilase time is normal in these patients.

Reptilase time

Reptilase is snake venom that cleaves only fibrinopeptide A from fibrinogen (in contrast to thrombin, which cleaves both fibrinopeptide A and fibrinopeptide B) and results in fibrin clot formation. This assay is prolonged by hypofibrinogenemia and most dysfibrinogenemias but is not prolonged by heparin because the reptilase enzyme is not inactivated by AT or direct thrombin inhibitors.

Global hemostasis tests

Thromboelastography/thromboelastometry involves monitoring the viscoelastic properties of whole blood during clot initiation, stabilization, and lysis. Two commercial instruments: TEG (Haemonetics, Braintree, MA) and ROTEM (TEM International, Munich, Germany) are currently available in most geographies. The change in viscosity of blood as it clots in a cup is transmitted through

a pin immersed into the blood through a mechanical-electrical transducer, producing a tracing of clot firmness over time. Certain patterns correlate with coagulopathies, hypofibrinogenemia, thrombocytopenia, and hyperfibrinolysis. Most experience with viscoelastic testing has been in liver transplantation, trauma, and cardiopulmonary bypass surgical settings, where rapid point-of-care hemostasis information is used to select appropriate blood component transfusion and factor replacement therapy.

Point-of-care (POC) hemostasis tests

There are a number of commercially available point-of-care (POC) coagulation devices that utilize whole blood samples and measure PT/INR, aPTT, and/or activated clotting time (ACT). These devices vary with regard to specimen volume requirements, active reagents, and endpoint detection methods, but have in common single-use test cartridges.

With the growing numbers of anticoagulation clinics and anticoagulation management services, patient self-testing and patient self-management with POC PT/INR testing has increased. However, POC devices which determine a thromboplastin-initiated clotting time that is electronically converted to a PT and/or INR, have limitations in accuracy and precision when compared to laboratory-based methods. These limitations include incorrect calibration of the ISI to the World Health Organization standard, extrapolated mean normal PT, and nonlinearity at supratherapeutic levels. While the evidence does not support widespread use of POC INR testing in general practice, patient self-testing and patient self-management have been associated with improved anticoagulation control and decreased incidence of thromboembolic or major bleeding events.

Clinical applications of POC aPTT testing includes monitoring low-dose heparin therapy because UFH levels greater than 1 unit/mL may infinitely prolong the aPTT. As a result, the ACT, which measures the time in seconds from the activation of factor XII to the formation of a fibrin clot, remains the predominant test to manage UFH anticoagulation during interventional cardiac and vascular procedures, and during cardiopulmonary bypass. ACT assays use different activators (celite, kaolin) and are optimized for specific heparin ranges, from low-dose heparin concentrations such as those used in extracorporeal life support, to high-dose heparin therapy used in cardiac surgery. In addition, the ACT is impacted by other factors including thrombocytopenia, platelet dysfunction, hemodilution, hypofibrinogenemia, coagulation factor deficiencies, LACs, and hypothermia.

von Willebrand factor assays

Endothelial cells and megakaryocytes synthesize VWF, which undergoes dimerization and subsequent linkage

to form VWF multimers before secretion into the blood. Once released, large multimers undergo remodeling to smaller molecules via cleavage by the protease adisintegrin and metalloprotease with thrombospondin (ADAMTS13). VWF has multiple domains with specific functions to support its 2 activities: adhesion to connective tissue and platelets and binding factor VIII. Although most deficiencies of VWF are congenital, VWD can also be acquired—a condition known as acquired von Willebrand syndrome, which is often associated with lymphoproliferative disorders, particularly monoclonal gammopathy of unknown significance, autoimmune disorders, hypothyroidism, and severe aortic stenosis, as well as with ventricular assist devices. Laboratory testing for suspected VWD is challenging because of the variability of personal and family bleeding histories, multiple types of VWF defects, physiologic variables affecting VWF levels, and analytical imprecision of certain VWF test methods. Repeated testing is indicated to confirm abnormal results before diagnosing a patient with VWD. See Chapter 10 for additional information regarding clinical presentation, classification, and management of VWD.

Initial testing for von Willebrand disease

Global tests of primary hemostasis, including bleeding time and PFA-100/200 closure times, lack both sensitivity and specificity for VWD, and the aPTT is an indirect and potentially insensitive screening test for low factor VIII activity. VWF antigen concentration (VWF:Ag), VWF activity, VWF binding to collagen (VWF:collagen binding activity), and factor VIII activity measurements are sufficient initial screening tests. Reference intervals for these analytes vary based on blood type, with type O individuals having mean values approximately 25% lower than non-type O controls. Some laboratories provide blood type-specific reference intervals, whereas other laboratories provide a single reference range (with lower limits of approximately 50%) and note that asymptomatic type O individuals may have VWF antigen, VWF activity, and factor VIII levels as

low as 35% to 40%. It is reasonable to consider VWF levels in the range of 30% to 50% as risk factors for mild bleeding tendency and not necessarily as an indication of inheritable disease. Fluctuations of VWF in patients during physiologic alterations associated with acute stresses, the menstrual cycle, or pregnancy make the interpretation of these analytes problematic, and patients may require repeat testing. Several equivalent and accurate methods can be used to quantify VWF:Ag. Measuring VWF activity is another matter. The most widely used method and the current gold standard is the ristocetin cofactor assay (VWF:RCo), which can be performed by automated immunoturbidity assays using lyophilized platelets and ristocetin or by platelet aggregometry, and assesses VWF binding to the platelet GPIb/IX/V complex. Ristocetin, an antibiotic, binds to VWF causing a conformational change that mimics the effect of high shear stress *in vivo* to expose the platelet-binding domain. The VWF:RCo activity is sensitive to both quantitative deficiencies of VWF (type 1 and type 3 deficiencies) and to mutations causing reductions in high- and intermediate-weight VWF multimers or defects in platelet binding (types 2A, 2B, and 2M VWD). A VWF:RCo/VWF:Ag ratio of <0.7 supports a qualitative, or type 2 VWF defect and warrants specialized confirmatory testing (Tables 12-11 and 12-12; see video in online edition). The VWF:RCo assay is labor intensive, poorly standardized, and imprecise, leading to the development of alternative methods to assess adhesive activity, including ristocetin-induced binding to recombinant wild type GPIb fragments (VWF:GPIbR), spontaneous binding of VWF to a gain of function mutant GPIb fragment (VWF:GPIbM), or by binding of a monoclonal antibody to a VWF A1 domain epitope (VWF:Ab). Moreover, ristocetin binding site polymorphisms have been described and may affect the measurement of VWF activity by VWF:RCo.

Specialized testing to classify von Willebrand disease

Dismissing a diagnosis of VWD or confirming a diagnosis of type 1 or type 3 VWD usually can be accomplished

Table 12-12 Assays for VWD classification

VWD type	VWF activity	VWF antigen	RIPA	FVIII	Multimers
Type 1	↓	↓	↓	↓	Nl pattern
Type 2A	↓↓	↓	↓↓	↓	↓ High and intermediate
Type 2B	↓↓	↓	↑↑↑	↓	↓ High
Type 2M	↓↓	↓	↓↓	↓	Nl
Type 2N	Nl	Nl	Nl	↓	Nl
Type 3	↓↓↓	↓↓↓	↓↓↓	↓↓↓	Undetectable

Nl, normal; RIPA, ristocetin-induced platelet aggregation.

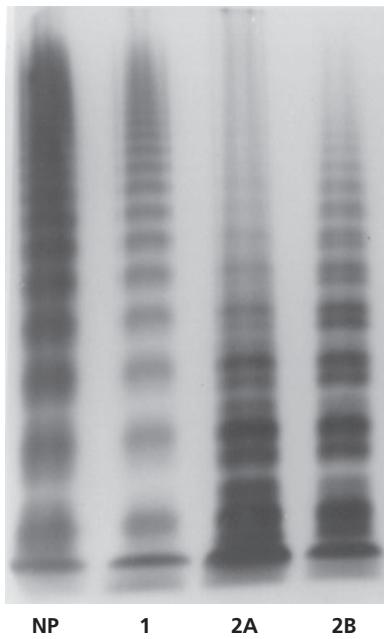


Figure 12-7 von Willebrand multimer patterns. NP, normal plasma; 1, type 1 VWD with normal bands but decreased staining intensity; 2A, type 2A VWD with loss of high and intermediate multimers; 2B, type 2B VWD with loss of high-molecular-weight multimers.

by reviewing VWF:Ag, VWF:RCo, and factor VIII activity results. A VWF:RCo or factor VIII activity result much lower than VWF:Ag is an indication for more specific testing.

VWF multimer analysis provides qualitative information by identifying structural abnormalities that correlate with qualitative defects in VWF adhesion (Figure 12-7). Electrophoresis of plasma through low-concentration agarose gel separates VWF multimer bands by size, which are detected with radio-labeled, enzyme-linked, or fluorescent VWF antibodies. Analysis of the band patterns can distinguish normal or subtly abnormal patterns (consistent with type 1 and 2N or 2M VWD, respectively) from major losses of high- and intermediate-size bands (consistent with type 2A, type 2B, and platelet-type VWD).

The ristocetin-induced platelet aggregation assay is a variation on the VWF:RCo assay to investigate platelet adhesion defects. Several ristocetin concentrations (ranging from 0.6 to 1.5 mg/mL) are added to separate aliquots of a patient's platelet-rich plasma. A change in light transmission is monitored by an aggregometer as platelets bind to VWF and aggregate (Figure 12-8). Normal and mild type 1 VWD platelet-rich plasma typically produces no or minimal aggregation at low ristocetin concentrations and increasing aggregation at higher concentrations. Platelet-

rich plasma from severe type 1 and types 2A and 2M VWD patients produces attenuated aggregation at high ristocetin concentrations, whereas platelet-rich plasma from type 2B or platelet-type VWD patients shows an enhanced aggregation response to low ristocetin concentrations. Estimates of the relative frequency of type 2B VWD to platelet-type VWD range from 8:1 to 10:1. Although the disorders have similar clinical presentations and inheritance is autosomal dominant, they require different types of hemostasis replacement products (VWF concentrate versus platelet transfusion, respectively). Mixing studies using normal washed platelets plus patient plasma, or vice versa, can distinguish whether the patient's VWF or platelet receptor is abnormal. In addition, some reference laboratories perform platelet-VWF binding assays using a VWF monoclonal antibody to assess the ability of a patient's VWF to bind formalin-fixed platelets in the presence of low-dose ristocetin. Genotyping to detect known mutations associated with each disorder is offered by a few reference laboratories.

Rarely, men and women with mild or moderate factor VIII deficiencies lacking X-linked inheritance pattern consistent with hemophilia A may be homozygous for type 2NVWD (decreased VWF binding affinity for factor VIII) or be compound heterozygous (type 1/2N). Decreased binding of recombinant factor VIII to the patient's immobilized VWF in an enzyme-linked immunoassay (ELISA) and equivalent VWF:Ag and VWF activity results are consistent with type 2N VWD. Genotyping specific for type 2N mutations is offered by a few reference laboratories.

Bleeding disorders with normal screening hemostasis tests

Abnormal, typically delayed bleeding due to severe factor XIII deficiency or fibrinolytic pathway defects are rare, yet should be considered when evaluations for coagulopathies and primary hemostasis defects are negative. Thrombin activates factor XIII, and factor XIIIa cross-links fibrin monomers to produce a durable clot. The urea clot lysis test is a qualitative screening test for severe factor XIII deficiency. Thrombin is added to plasma, and the clotted fibrin is added to a high-molar solution of urea that disrupts the clot if fibrin has not been cross-linked by factor XIIIa. Alternative quantitative assays are available to directly quantify factor XIII antigen and activity.

Global screening tests of the fibrinolytic system include the euglobulin clot lysis time (ELT), which measures the time to lyse a fibrin clot in the absence of plasmin inhibitors, and the whole blood clot lysis time (see the section "Global hemostasis tests" in this chapter). Congenital

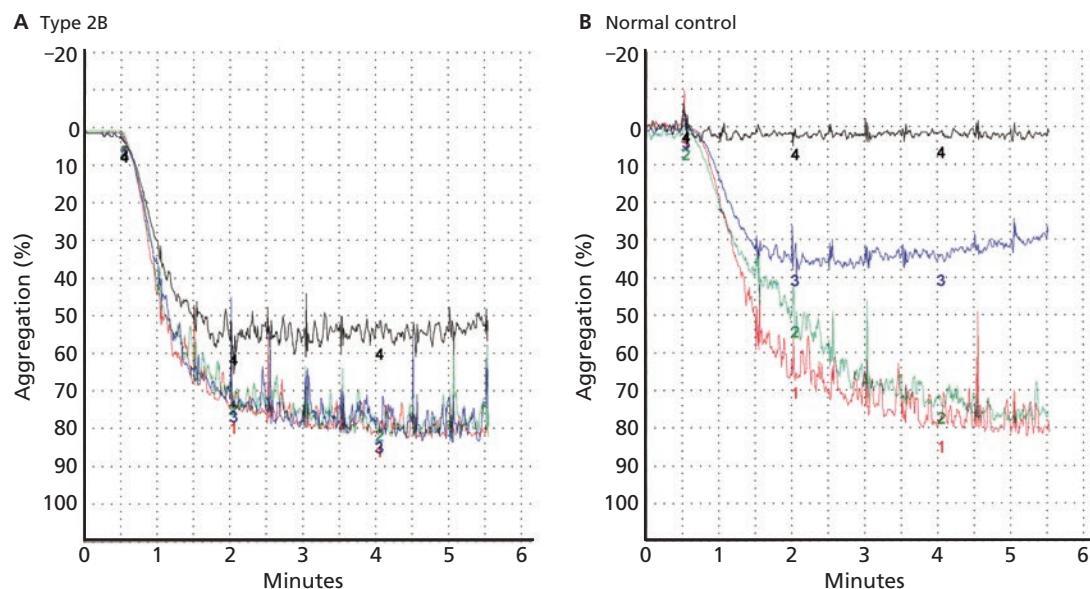


Figure 12-8 Examples of platelet-rich plasma aggregation responses to a range of ristocetin concentrations (1: 1.5 mg/mL, 2: 1.2 mg/mL, 3: 0.9 mg/mL, 4: 0.6 mg/mL). (A) Type 2B VWD patient showing >50% aggregation with all ristocetin concentrations; (B) normal control demonstrating concentration-dependent aggregation.

hyperfibrinolysis is due to deficiencies of natural plasmin inhibitors, plasminogen activator inhibitor 1 (PAI-1) and α_2 -antiplasmin, and laboratory evaluation requires a panel of analytes, including plasminogen, PAI-1 activity and antigen, tissue plasminogen activator (tPA) antigen, and α_2 -antiplasmin activity typically performed in reference laboratories. Causes of acquired hyperfibrinolysis resulting in circulating plasmin overwhelming α_2 -antiplasmin inhibition include decreased hepatic clearance of tPA due to advanced cirrhosis or during liver transplantation, increased release of tPA from endothelial cells during cardiopulmonary bypass, amyloidosis, envenomization from several species of snakes, and as a component of DIC associated with acute promyelocytic leukemia and rarely with solid tumors, including bladder or prostate cancer. Laboratory evidence for primary fibrinolysis includes reduced fibrinogen levels due to cleavage by plasmin, elevated fibrin(ogen) degradation products, and no significant elevation of D-dimer levels because lysis of cross-linked fibrin clot is not the dominant process. DIC is the result of a primary disease process that leads to the release of tissue factor or other coagulation-activating factors into the blood. Because of variations in the amount and rate of procoagulant material released determined by the underlying disease, there are no diagnostic patterns of laboratory results. In acute, overwhelming DIC, initial platelet counts and fibrinogen levels are low, or serial testing shows a downward trend. PT, aPTT, and TT may be prolonged,

depending on the severity of consumption, and D-dimer levels are markedly elevated, indicating unregulated thrombin activity and secondary fibrinolysis.

Vessel wall defects, such as collagen diseases (eg, Ehlers-Danlos and Marfan syndromes), also can cause abnormal bleeding. In addition to physical examination and imaging information, genetic testing is becoming more readily available for some of these syndromes.

Heparin monitoring

Most hospitals use aPTT-based nomograms to guide UFH anticoagulation; however, monitoring heparin anticoagulation with the chromogenic anti-Xa assay is the preferred approach in some hospitals as an alternative to aPTT. Advantages of using anti-Xa for UFH monitoring include: a shorter time to a therapeutic result; less variability resulting in decreased dosage changes and ordered tests; no confounding from factor deficiencies, LACs, or acute phase reactants; and limited interferences from common biologic substances. In addition, anti-Xa can be used when a patient's baseline aPTT is prolonged because of an LAC or deficiency of a contact activator (XII, PK, or HMWK).

The anti-Xa assay is a variation of a chromogenic AT assay (see section on assays for thrombophilia) comparing an unknown concentration of heparin in the patient plasma to a calibration curve prepared with a UFH, LMWH, or hybrid curve. Following addition of activated factor Xa to the test plasma, the rate of factor

Xa neutralization by AT is positively correlated with the heparin concentration, and the rate of chromogenic substrate cleavage by factor Xa is inversely correlated with the heparin concentration.

LMWHs may minimally prolong the aPTT at therapeutic concentrations. LMWHs typically do not require monitoring. However, under certain situations, including patients of extremely low and high body weight, pediatric patients, pregnant patients, and patients with impaired renal function, monitoring peak plasma LMWH activity (approximately 4 hours after a subcutaneous injection) using a chromogenic anti-Xa assay is recommended.

Platelet function tests

In vitro assessment of platelet activation and aggregation in response to selected platelet agonists should be reserved for patients with convincing bleeding histories in whom evaluations for coagulopathies, VWD, and moderate-to-severe thrombocytopenia are negative. In addition, prescribed and over-the-counter medications that can inhibit platelet function must be discontinued before testing. Many disease processes can produce acquired qualitative platelet defects, including uremia, liver failure, and myeloproliferative and myelodysplastic disorders, but formal aggregation studies are usually not informative in these cases. Platelet function testing is technically demanding, time consuming, and poorly standardized, even despite recent guidelines for performing and interpreting these studies. The hematologist should be aware that labs use different platforms to analyze platelet aggregation: instruments that are used to test platelet-rich plasma (light transmission aggregometry) and instruments that use whole blood (whole blood aggregometry). Testing is performed on aliquots of citrated whole blood or platelet-rich plasma with different concentrations of agonists, such as adenosine diphosphate (ADP), epinephrine, and collagen; arachidonic acid, which platelets metabolize to the agonist thromboxane A₂ via the cyclooxygenase pathway; and ristocetin to screen for platelet GPIb/IX/V deficiency. Formation of platelet aggregates causes an increase in light transmission over time. Figure 12-9 shows a normal aggregation response of platelet-rich plasma to collagen and ADP, and a clear first and second wave with epinephrine, indicating initial aggregation in response to exogenous epinephrine followed by additional, irreversible aggregation because of a release of ADP from platelet-dense granules. The platelet release reaction can be assessed in a lumi-aggregometer, which simultaneously monitors whole blood aggregometry through changes in electrical impedance as platelets aggregate and platelet activation when released adenosine triphosphate combines with luciferin/luciferase enzyme-

releasing light. Certain patterns of platelet aggregation responses to a panel of agonists are sensitive to specific inherited and rare qualitative platelet disorders, including Glanzmann thrombasthenia, Bernard-Soulier syndrome, and collagen receptor defects. Platelet secretion defects resulting from abnormal signal transduction and qualitative and quantitative granule disorders are more common, produce variable aggregation patterns, and require additional diagnostic tests that are not readily available for clinical use. These tests, including platelet electron microscopy, may be accessible through research or reference laboratories.

Global primary hemostasis screening tests

The template bleeding time is an invasive test, fraught with difficult-to-control technical and patient variables, and lacks sensitivity and specificity for detection of primary hemostasis disorders. Prolonged bleeding times performed on asymptomatic patients do not predict a risk of abnormal bleeding during surgery or other invasive procedures. The test is performed by making a standard incision in the forearm using a spring-loaded blade while maintaining a blood pressure cuff at 40 mm Hg. Blood oozing from the incision is wicked away with filter paper every 30 seconds until bleeding stops. The typical reference range in adults is approximately 5 to 10 minutes.

Most laboratories have discontinued performing bleeding times and substituted automated *in vitro* screening methods, which do not require an incision and provide more precise results from samples of blood collected in citrate, yet have similar limitations. The PFA-100/200 instrument monitors VWF-dependent platelet adhesion and aggregation under conditions that mimic the shear forces in the arterial circulation. Citrated blood is aspirated through a minute aperture in a membrane coated with collagen and ADP (COLL/ADP) or collagen and epinephrine (COLL/EPI). VWF multimers bind to collagen and platelets adhere to VWF, are activated by COLL/ADP or COLL/EPI, aggregate, and occlude the aperture, which is recorded as closure time in seconds. Each laboratory must determine reference intervals, although typical ranges are 55 to 137 seconds and 78 to 199 seconds for COLL/ADP and COLL/EPI cartridges, respectively. Prolonged PFA-100/200 closure times are not sufficiently sensitive for all congenital qualitative platelet disorders and types of VWD to be used as a general screening test. In addition, as anemia and thrombocytopenia worsen, closure times increase, and these variables should be considered when interpreting prolonged closure times in the setting of hematocrit <30% and platelet count <100 × 10⁹/mL.

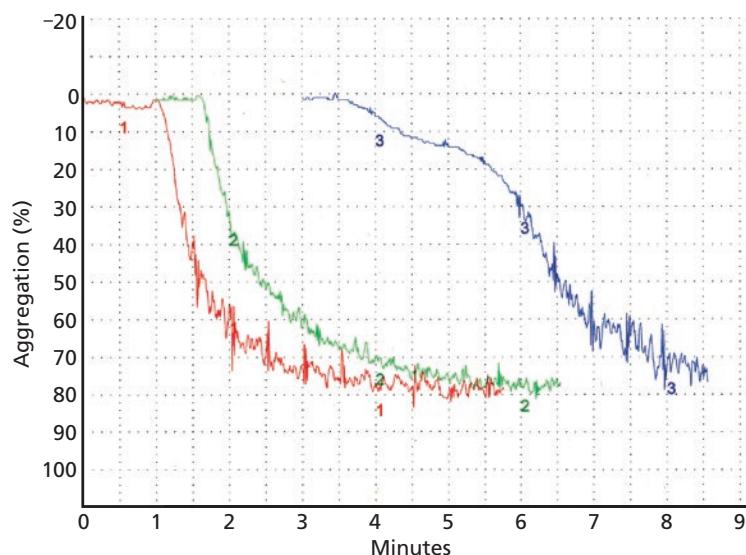


Figure 12-9 Representative platelet aggregation curves performed on normal platelet-rich plasma. 1: collagen, 5 mg/mL; 2: ADP, 5 mg/mL; 3: epinephrine, 5 μ M.

Prolonged COLL/EPI closure time is a sensitive test for aspirin inhibition of platelets, but the COLL/ADP closure time is insensitive to blockade of the platelet P2Y12 ADP receptor by thienopyridines.

Specialized testing for acquired thrombocytopenia

Assays for platelet antibodies

Immune-mediated thrombocytopenia remains a clinical diagnosis of exclusion due to the general poor performance of laboratory methods to detect platelet-specific antibodies. Assays detecting total or surface-bound platelet immunoglobulins are nonspecific and are not recommended.

Assays for heparin-induced thrombocytopenia

HIT is a clinical diagnosis supported by serologic and functional assays. *In vitro* functional assays monitor activation of control platelets by patient serum in the presence of therapeutic concentrations of heparin and at high heparin concentrations. Activation with a low heparin concentration and no activation at high heparin concentration are considered to be both specific and sensitive for detection of platelet factor 4 (PF4) heparin-immune complexes, which are capable of causing *in vivo* platelet activation, thrombocytopenia, and thrombosis. In North America, selective laboratories perform the serotonin release assay (SRA) to monitor carbon-14-labeled serotonin secretion from control platelets. In Europe, heparin-induced platelet activation assay performed in microtiter wells with visual detection of platelet aggregation is the preferred method.

Both assays are technically difficult, labor-intensive, and not readily available.

Commercial antigen assays (eg, ELISA) detect antibodies recognizing immobilized PF4 bound to heparin or polyvinyl sulfonate complex. Although sensitive, HIT ELISA results are nonspecific, detecting antibodies incapable of activating platelets *in vitro* or causing thrombocytopenia and thrombosis *in vivo*. The PPV of a positive PF4 ELISA result alone to confirm a diagnosis of HIT is low, and if used as the only criterion, a positive PF4 ELISA results in the overdiagnosis of HIT. Growing evidence supports several approaches to improving the specificity of PF4 ELISA testing. First, clinicians can improve the pretest likelihood that thrombocytopenia is due to HIT by applying a validated clinical scoring system such as the 4Ts (thrombocytopenia, timing, thrombosis, and exclusion of other more likely causes of thrombocytopenia). Patients with low 4T scores are unlikely to have HIT, even with a positive PF4 ELISA, removing the need for testing. This is especially true for patients who have an increased likelihood of having an FP test, such as patients who have recently had cardiopulmonary bypass. Second, identifying only IgG instead of a combination of IgG/IgM/IgA PF4/heparin antibodies improves the specificity of a positive PF4 ELISA with a slight impact on sensitivity. Finally, ample evidence suggests that the higher a HIT ELISA optical density (OD) is, the more likely a functional HIT assay will be positive and the clinical presentation and course will be consistent with HIT. No cutoff point, however, completely segregates all platelet-activating antibodies from

nonactivating antibodies. Conversion from viewing HIT ELISA results as simply positive or negative to considering OD as a continuous variable, with increasing probability for HIT as OD increases, is still evolving as clinical research continues.

For rapid detection of PF4/heparin antibodies, additional assays such as the particle gel immunoassay (PaGIA), particle immunofiltration assay, latex agglutination assay, and chemiluminescent immunoassays have been added to the armamentarium of HIT testing. With analytical turnaround times less than 30 minutes and on-demand availability, these assays allow clinicians to make an informed decision before switching to alternative anticoagulation. Recently, the latex agglutination assay was evaluated in 429 patients from a prospective cohort study of 4Ts scoring and consecutive HIT patients at a single institution using reference SRA. The authors demonstrated a high NPV (99.7%) and PPV (55.6%), and a diagnostic specificity and PPV higher than that of 2 ELISAs and PaGIA. A similar evaluation was recently published for the chemiluminescent assay; the IgG-specific chemiluminescent assay had a high combination of sensitivity and specificity (98.8% and 98.5%, respectively) relative to other immunoassays. Unlike other antigen assays, the latex and chemiluminescent immunoassays are fully automated and standardized with a monoclonal antibody calibrator, which allows for the possibility of comparable test results from different laboratories.

Assays for TTP and VWF-cleaving protease (ADAMTS13)

In sporadic cases of TTP, ultralarge forms of VWF initiate the formation of platelet aggregates and lead to thrombi and thrombocytopenia. In these cases, the activity of the VWF-cleaving protease, ADAMTS13, typically is low (ie, <10%), and in many cases, *in vitro* evidence of an inhibitory autoantibody is present. In hereditary forms of TTP, there are mutations in the gene encoding the enzyme, and the activity of ADAMTS13 is absent or markedly decreased; however, no inhibitor is present.

The main laboratory methods that are currently used employ a recombinant 73-amino-acid peptide from the A2 domain of VWF containing the Y1605-M1606 bond recognized by ADAMTS13 to detect substrate cleavage by either ELISA, fluorescence resonance energy transfer (FRET), or chemiluminescent methods. Two amino acids in the peptide substrate are modified in the FRET assay; one fluoresces when excited, and the other absorbs or quenches the released energy. When ADAMTS13 cleaves the substrate and separates the modified amino acids, emitted energy is detected in a fluorescent plate reader. The

method for ADAMTS13 neutralizing antibody detection is similar to the Bethesda assay for factor VIII inhibitors; dilutions of patient serum or plasma are mixed with PNP followed by measurement of residual enzyme activity using the synthetic substrate. Typical reference values are ADAMTS13 activity >67% and inhibitor titer <0.4. Measuring ADAMTS13 antigen is not necessary when evaluating a patient for sporadic or idiopathic TTP.

The decision about whether to initiate plasma exchange is made on the basis of clinical assessment and should not be delayed by ADAMTS13 testing in the absence of a rapid test. Importantly, samples for assessment of ADAMTS13 activity and inhibitor should be obtained prior to transfusion with fresh frozen plasma or plasma exchange; however, samples may also be obtained prior to apheresis treatments if a pre-apheresis sample is not available. ADAMTS13 testing may be obtained following completion of a course of plasma exchange because persistently low ADAMTS13 activity and positive inhibitor titers are predictors of relapse during remission.

Assays for thrombophilia

Inherited deficiency of 1 or more of the identified natural inhibitors of coagulation (AT, PC, and PS) is a risk factor for venous thrombosis, and functional and immunologic assays are available to measure these inhibitors. The use of these assays generally should be restricted to patients in whom the result may affect prognosis and duration of anticoagulant treatment. This generally includes patients who present with spontaneous thrombosis not temporally related to recent surgery, trauma, immobilization, cancer, or other acquired risk factors. The likelihood of identifying a deficiency is increased if thrombosis is recurrent or in an unusual location, the patient is young (<45 years old), or the patient has a positive family history of thrombosis. To avoid misleading low results due to temporary conditions related to acute illness, thrombosis, and anticoagulant therapy, testing for non-genetic-based assays ideally should be delayed until several weeks after completion of treatment when a patient has returned to baseline. The biologic and analytical variability associated with phenotypic diagnoses of these deficiencies requires verification of an abnormal test result on a new sample. Because of the large number of mutations associated with deficiencies of AT, PC, and PS, genotyping is not routinely performed.

Antithrombin deficiency

The most sensitive screening tests for AT deficiency are chromogenic activity assays designed to quantify AT inhibition of factor Xa or IIa in the presence of UFH.

Abnormal low AT activity results should be repeated and may be followed by the measurement of AT antigen to classify the deficiency as type I (activity = antigen) or type II (activity < antigen); however, the clinical significance of subclassification is unclear. Type I AT deficiency is more common than type II deficiency in symptomatic kindreds. Subclassification of type II deficiency requires performance of the chromogenic activity assay without heparin to differentiate type IIa resulting from reactive site defects and IIb resulting from AT heparin-binding defects. Although type IIb is associated with a low risk of thrombosis, progressive AT activity assays are not readily available and typically not performed.

Protein C deficiency

The preferred screening test for PC deficiency is a chromogenic activity assay. PC is activated with a snake venom and PC activity correlates with hydrolysis of a synthetic peptide and change in OD. Clot-based PC activity assays are an alternative, but potentially inaccurate results may occur due to variations in factor VIII and PS levels, FVL, inhibitory antibodies, and presence of some anticoagulants. Abnormal low PC activity results should be repeated and may be followed by measurement of the PC antigen to classify the deficiency as type I (activity = antigen) or type II (activity < antigen); however, the clinical significance of subclassification is unclear.

Protein S deficiency

PS assays are challenging because of the unique biology of PS. Total plasma PS is partitioned between free and bound forms. The protein is nonfunctional when bound to complement 4b-binding protein and functional when it is free. In its unbound form, the protein can serve as a cofactor for activated PC (aPC). The typical PS bound-to-free ratio of 60:40 varies under different physiologic and pathologic conditions. Clot-based PS activity assays are the most sensitive screening tests for PS deficiency but suffer from potential inaccuracy because of the same variables that can affect PC activity testing. An alternative screening assay is free PS antigen concentration to avoid confounding variables. Free PS testing, however, is insensitive to type II PS deficiency (low activity but normal free antigen level). Some laboratories screen with PS activity, some screen with free PS antigen, and other laboratories use both assays.

Factor V Leiden and prothrombin gene mutations

Two autosomal inherited coagulation factor variants increase the risk for VTE; these are factor V G1691A (FVL) and prothrombin G20210A. Several sensitive commer-

cial clot-based screening assays for FVL mutation demonstrate a resistance of factor Va cleavage by aPC in the presence of FVL mutation. Coagulation testing, activated with aPTT, PT, or Russell's viper venom reagents, is performed with or without added aPC, and the clotting times are expressed as a ratio. Abnormally low ratios represent aPC resistance (aPCr). Specificity is improved by repeat testing of positive plasmas after dilution with factor V-depleted plasma to minimize impact of inhibitors, anticoagulants, and high factor VIII levels. Genotyping should be performed on all aPCr-positive patients to determine whether they are heterozygous or homozygous for FVL. Although prothrombin G20210A mutation is associated with elevated prothrombin levels, measuring factor II activity is not a sensitive screening test, and genetic testing is the primary method.

Antiphospholipid syndrome

APS is an important acquired thrombotic condition. Consensus-based criteria have been developed for the investigational classification of APS. These criteria require a combination of clinical conditions (unexplained venous or arterial thromboembolic events, pregnancy morbidity) and persistent laboratory evidence of autoantibodies that recognize epitopes on selected proteins associated with phospholipids and identified by coagulation-based (LACs) or serologic-based (aCL and anti-β₂-glycoprotein IgM and IgG antibodies) testing. LACs are heterogeneous antibodies that interfere with *in vitro* clotting assays. Indirect evidence for the presence of an LAC requires: (i) prolongation of a screening clotting assay designed to be sensitive to the phospholipid-dependent behavior of LAC, (ii) ruling out prolongation due to a coagulopathy by showing incomplete correction in a 1:1 mix of patient and normal pooled plasma, and (iii) confirming phospholipid dependence by shortening the clotting time with the addition of more phospholipid. Although some LACs are discovered when a routine aPTT is prolonged, a normal aPTT is generally not a sensitive LAC screening test and should not prevent performance of more sensitive LAC testing based on the clinical circumstances. There is no gold-standard LAC method. Recent updated consensus expert guidelines from the International Society of Thrombosis and Hemostasis Scientific Subcommittee on Lupus Anticoagulant/Phospholipid Antibodies and Clinical and Laboratory Standards Institute recommend performing 2 sensitive LAC tests in parallel—one aPTT-based test and one Russell's viper venom (activation of factor Xa)-based test—and accepting a positive result from either or both as evidence of an LAC. Preanalytical variables requiring attention include platelet contamination (>10,000/mL)

due to inadequate centrifugation, which can produce FN LAC results because of the neutralizing effect of platelet-derived phospholipid, and concurrent anticoagulation therapy. The presence of a direct thrombin inhibitor or factor Xa inhibitor in the test plasma nullifies the validity of LAC testing. Heparin can be neutralized by additives in the LAC test reagents or in a separate step before testing, and the mixing step can compensate for mild to moderate coagulopathies due to liver disease or vitamin K antagonists like warfarin. The preferred time, however, for LAC testing is before or after anticoagulation treatment. Rarely, a specific factor inhibitor can cause an FP LAC result, typically with an aPTT-based LAC test due to a factor VIII inhibitor. A more frequent occurrence, however, is the appearance of multiple coagulation factor deficiencies when the true coagulation factor levels are within normal limits; this misleading picture occurs because the same antibodies responsible for the LAC effect also interfere with coagulation factor assays. The hematologist should be aware that rare patients concurrently may have both an LAC and a true factor VIII inhibitor. Abnormal bleeding likely would be present, and specific factor assays would confirm an isolated factor deficiency. LAC tests are either positive or negative, and evidence is insufficient to support reporting gradations of positive results. Because of differences in test methods, reagents, instrumentation, preanalytical variables, and approaches to analyzing and reporting results, there is substantial interlaboratory variability of LAC results based on external proficiency testing surveys.

LAC can cause reagent-dependent prolongations of PT results. Although this is usually mild, occasionally LAC-positive patients have elevated INRs before starting warfarin. Chromogenic factor X activity (not chromogenic anti-Xa) is an alternative to the INR for therapeutic anticoagulation monitoring (target 20% to 40%); however, availability of the test is limited. Another option is to measure PT-based factor II, VII, and X activities and observe whether the LAC produces an inhibitor pattern on the serial dilutions of plasma. If 1 or more factor assays appear unaffected by the LAC, then suppression of a specific clotting factor can serve as the therapeutic target for warfarin anticoagulation. A markedly prolonged PT in the setting of LAC may be a result of acquired factor II deficiency due to a nonneutralizing prothrombin auto-antibody that increases the clearance rate. These patients are at risk for spontaneous bleeding. To recognize this rare condition, a factor II activity level should be obtained in an LAC-positive patient with a prolonged PT/INR.

Performance of immunoassays for aCL and anti- β_2 -glycoprotein I ($\alpha\beta_2$ GPI) IgM and IgG antibodies should

accompany LAC testing to maximize sensitivity because persistently positive (arbitrarily defined as >12 weeks apart) results from serologic tests or LAC, or both, fulfill the laboratory criteria for APS. Commercial ELISA kits and chemiluminescent assays for aCL and $\alpha\beta_2$ GPI lack standardization, and interlaboratory agreement is poor for weakly positive sera. To improve specificity, some experts consider only medium- and high-titer-positive IgG and IgM aCL and $\alpha\beta_2$ GPI results to be clinically important. In addition, significant immunosuppression (especially humoral) may lead to FN results. While other antiphospholipid antibody specificities are currently not included in the classification criteria, antibodies to $\alpha\beta_2$ GPI domain I and antiphosphatidylserine/prothrombin antibodies have been shown to be predictive of thrombotic risk.

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Transfusion medicine

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Introduction

Transfusion medicine encompasses blood collection, pretransfusion compatibility testing, transfusion of blood components for the appropriate indications, and recognition and evaluation of adverse reactions to transfusion. Specific hematology populations, such as patients with sickle cell disease (SCD) and hematopoietic stem cell transplant (HSCT) recipients, pose unique transfusion-related challenges that are of particular relevance to hematologists. Apheresis is another integral component of transfusion medicine that includes therapeutic apheresis, which removes or modifies a constituent of whole blood contributing to disease pathogenesis, peripheral blood stem cell (PBSC) harvesting for allogeneic or autologous HSCT, and mononuclear cell harvesting for donor lymphocyte infusion or engineered cell therapy.

Red blood cell (RBC) transfusion

The ABO system

The ABO system is the most clinically relevant blood group system in transfusion and transplantation medicine. The ABO system is a group of carbohydrate antigens defined by their terminal saccharide moiety. The subterminal galactose, in association with a constitutively expressed fucose moiety, defines the H antigen. The addition of N-acetylgalactosamine or galactose to the subterminal galactose yields red blood cells (RBCs of group A or group B, respectively). Individuals who express both sugars are group AB, whereas individuals who express neither of these sugars are group O. As the H antigen remains unmodified in these group O individuals, some authors refer to the ABO antigen system as the ABH system. Blood group O is most common in the white U.S. blood donor population (45%), followed by group A (40%), group B (11%), and group AB (4%). In African Americans, the order of frequency is similar, but there are fewer group A (27%) and more group B (20%).

The ABO gene is on chromosome 9, spans over 18 kilobases, and consists of 7 exons. The A and B genes encode transferase enzymes that covalently attach the specific terminal saccharide moiety to the subterminal galactose. Serologic typing for ABO is simple, fast, and inexpensive. The large number of ABO alleles (>350) has precluded the routine use of genotyping methods for ABO blood group pre-

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diction. The group O phenotype is an autosomal recessive trait, representing inheritance of 2 nonfunctional ABO genes. Individuals with blood group O have lower levels of von Willebrand factor (VWF), and in the past was taken into consideration in the diagnosis of mild type I von Willebrand disease. Conversely, nongroup O-individuals have a greater risk of venous thromboembolism, potentially attributable to higher levels of VWF and FVIII.

Healthy individuals past infancy produce immunoglobulin M (IgM) anti-A or anti-B antibodies, also known as isoantibodies, directed against the respective ABO antigens that are not present on their own cells. Thus, group O individuals have so-called naturally occurring anti-A and anti-B antibodies, group A individuals have anti-B antibodies, group B individuals have anti-A antibodies, and group AB individuals have neither. ABO compatibility is the most important factor in determining whether blood from a specific donor can be transfused to a specific recipient. Preformed recipient isoantibodies predictably induce acute hemolysis if ABO-incompatible RBCs are transfused. Because anti-A and anti-B isoantibodies are predominantly of the IgM isotype, and thus efficient at fixing complement, the ensuing hemolysis is intravascular and can be severe—leading to shock, renal failure, disseminated intravascular coagulation (DIC), and death. In blood group O individuals, an additional antibody, anti-A,B, which cross-reacts with both type A and type B red cells, is also present and predominantly of the immunoglobulin G (IgG) isotype. Because IgG antibodies may cross the placenta, whereas IgM antibodies cannot, the presence of IgG isoantibodies in blood group O individuals explains the higher frequency of ABO hemolytic disease of the fetus and newborn (HDFN) in blood group O mothers with non-blood group O fetuses and newborns.

ABO subgroups differ in the amount of A and B antigen expressed on the RBC and are occasionally clinically significant. The most common subgroups identified in routine testing are A₁ and A₂, which differ in their glycosyltransferase enzyme activity, resulting in quantitative and qualitative differences in A antigen expression. The majority of group A individuals are subtype A₁ (80%). Type A₂ individuals express substantially less A antigen and 1% to 8% of type A₂ and 22% to 35% of A₂B individuals have alloanti-A₁ in their sera. Anti-A₁ can cause ABO discrepancies during routine testing and lead to incompatible crossmatches with A₁ or A₁B red cells. Anti-A₁ antibodies typically bind only to A₁-positive RBCs at nonphysiologic temperatures, reacting best at room temperature or below. Anti-A₁ antibodies are only considered clinically significant if reactive at 37°C or at the antihuman globulin (AHG or Coombs) phase, and in these cases, A₂ or O red

cells should be transfused. Subgroups of B antigen exist as well but are encountered much less frequently.

ABO antigens are also expressed on endothelial cells. ABO compatibility is typically required for solid organ transplantation to avoid ABO antibody-mediated acute humoral rejection. A blood group O recipient transplanted with a solid organ from a group A donor is at risk of humoral rejection and destruction of the transplanted organ mediated by the recipient's anti-A antibodies. There are a few exceptions to the requirement for ABO compatibility in solid organ transplantation, most of which involve donors of the A₂ subgroup or infants who have not yet begun to produce isoantibodies. ABO compatibility is not required for hematopoietic stem cell (HPSC) transplantation because ABH antigens are not expressed on HPSCs and engraftment of HPSCs is not inhibited by circulating ABO antibodies. ABO incompatibility is present in many HPSC donor-recipient pairs, necessitating special attention to blood component selection for the recipient. Passenger lymphocyte syndrome may be seen in both solid organ and minor ABO-incompatible HPSC transplants. In these cases, passenger donor B-lymphocytes may continue to produce isoantibodies in the recipient and result in donor ABO antibody-mediated hemolysis of the recipient's RBCs. Transplantation of a liver from a group O donor would be acceptable for a group A recipient because the recipient's anti-B antibodies do not cause humoral rejection of the transplanted organ. However, if the solid organ contains passenger lymphocytes from the group O donor which are producing anti-A and anti-B antibodies, the anti-A antibody may cause hemolysis of the recipient's circulating type-A red cells. Transfusion of donor ABO-type RBC components mitigates passenger lymphocyte syndrome.

The Rh system

The Rh blood group system is highly immunogenic, complex, and polymorphic. Rh immunization occurs by pregnancy, transfusion, or stem cell transplantation. The *RH* locus is comprised of 2 homologous genes, *RHD* and *RHCE*, which encode the D antigen and the CcEe antigens in various combinations (ce, cE, Ce, and CE), respectively. More than 60 Rh antigens have been defined serologically and over 500 *RHD* and 150 *RHCE* alleles have been reported to date. The *RH* genes are 97% identical, include 10 exons, and evolved from a gene-duplication event on chromosome 1. The 2 proteins are 416 amino-acid, nonglycosylated transmembrane proteins that differ by 32 to 35 amino acids, depending on whether D is compared to ce, cE, Ce, or CE. Individuals who are referred to as "Rh positive" express the D antigen; approximately

85% of whites and 92% of blacks are D positive. Individuals who are “Rh negative” do not express D antigen, either because they have a complete deletion of the *RHD* gene, which is the most common cause in individuals of European descent, or have nonfunctioning *RHD* resulting from premature stop codons, gene insertions, or other causes that are common in Asian and African individuals. This magnitude of difference between the 2 Rh proteins may explain the relatively high degree of immunogenicity of the D antigen to the Rh-negative individual when compared with the immunogenicity of other blood group antigens in which single amino acid changes distinguish their polymorphic alleles.

Inheritance of the Rh_{null} phenotype is extremely rare, in which none of the Rh antigens are expressed on the RBC surface. The Rh_{null} phenotype most often results from mutations in *RHAG*, which encodes the Rhag protein that trafficks RhD and RhCE to the RBC membrane surface. The Rh_{null} phenotype caused by *RHAG* mutations is associated with stomatocytic erythrocytes and a low-grade hemolytic anemia. The Rh polypeptides D and CE are ammonia transporters and facilitate the assembly of major transport proteins in the RBC membrane, such as band 3. Less commonly, Rh_{null} individuals have mutations in the *RHCE* alleles in combination with the common *RHD* deletion.

Blood is routinely typed for RhD and D negative (D⁻) RBCs are provided to D⁻ individuals for 2 primary reasons. First, the D antigen is highly immunogenic and approximately 80% of D⁻ individuals become alloimmunized if exposed to D, resulting in hemolysis, although the risk appears to be lower in the setting of massive hemorrhage. Second, anti-D antibodies can cause significant HDFN. Prior to Rh(D)-immune globulin prophylaxis, anti-D frequently caused HDFN. RhIg is 99.9% effective in preventing maternal alloimmunization to D when administered to D⁻ females at 28 weeks of pregnancy (typically as a single dose of 300 mcg), and if the newborn is D⁺, a post-delivery dose is calculated based on the estimated volume of fetal-maternal hemorrhage. The exact mechanism by which RhIg prevents sensitization in the D⁻ individual when exposed to D⁺ RBCs remains unknown. One proposed mechanism is that D⁺ fetal RBCs coated with RhIg in the maternal circulation serve to cross-link surface immunoglobulin to inhibitory Fc receptors on maternal naïve B-cells to render them anergic. The other major antigens of the Rh system—C, c, E, and e—are also relatively potent immunogens and can cause HDFN of varying severity, albeit at lower frequencies than D. No immune globulin preparations are available for the prevention of alloimmunization to Rh antigens other than D.

The *RH* genes are highly polymorphic, particularly in specific ethnic backgrounds, including individuals of African descent. The close proximity of *RHD* and *RHCE*, their sequence homology, and opposite orientation has resulted in many variant and hybrid alleles evolving on both loci. Standard serologic Rh typing does not always detect the many Rh antigenic variations and genotyping is required for identification. Variant *RH* alleles encode weak and/or partial expression of D, C, c, E, and e. Partial antigens describe RBCs that lack 1 or more common epitopes associated with expression of the antigen. As a result, a transfusion recipient can produce antibodies to foreign Rh epitope(s) they lack. A D⁺ individual with partial D expression is at risk of anti-D with transfusion of D⁺ units, and if pregnant with a D⁺ fetus with foreign D epitopes inherited from the father, can experience HDFN. Therefore, such individuals should be given RhIg. These individuals are often identified only after they have formed anti-D despite typing as Rh positive, or may present with inconsistent D typing results with different reagents that recognize different epitopes of the D polypeptide. Once a patient has formed anti-D, RhD-negative RBCs are indicated. Providing RhD-negative RBCs prophylactically to these patients is not currently the standard of care but can be recommended on an individual case. For patients with weak expression of Rh, the antigenic density on the RBC surface is significantly reduced but all common epitopes are present. Individuals with weak D phenotypes can be considered Rh positive; that is, they are immunologically tolerant to D. Likewise, a pregnant woman with a weak D phenotype carrying an Rh-positive fetus would not need to receive RhIg to prevent D sensitization. DNA-based methods may be utilized to distinguish weak and partial D.

For blood donors, reagents and techniques to detect weak D expression are paramount so that donor units with weak or partial D antigens are labeled D⁺ to prevent anti-D immunization of D⁻ recipients. Blood collection centers are required to test donor blood for weak expression of D and label these donors as D⁺. Conversely, when the D type of a patient is determined, a weak D test is not required except to assess the RBCs of an infant whose mother is at risk of anti-D immunization. Most hospital blood banks choose D typing reagents and methods that do not detect weak D phenotypes when testing patients. Thus, an individual with weak D expression may be classified as D⁻ as a patient but D⁺ as a blood donor.

Other protein antigen blood group systems

Outside the ABO and Rh systems, most clinically significant blood group alloantibodies are directed against protein-based antigens, particularly antigens in the Kell, Kidd, Duffy,

Table 13-1 Commonly-occurring RBC antigens of clinical significance

RBC antigen system	Molecule expressing antigen	Function of molecule	Antibody immune/naturally occurring	Hemolytic transfusion reaction from antibody	HDFN from antibody
ABO	Glycoprotein or glycolipid	Unknown	Naturally occurring	Yes, acute	Yes, usually mild (IgG anti-A,B generally present in blood of group O mothers)
Rh	Protein	Ammonium ion transport	Immune	Yes, delayed	Yes, can be severe
Kell	Glycoprotein	Member of neprilisin (M13) family of zinc metalloproteases	Immune	Yes, delayed	Yes, often severe
Kidd	Glycoprotein	Urea transport	Immune	Yes, delayed	Yes
Duffy	Glycoprotein	Chemokine receptor DARC (Duffy antigen receptor for chemokines)	Immune	Yes, delayed	Yes
MNSs	Glycoprotein	Structural role in RBC membrane (glycophorins A and B)	Naturally occurring (anti-M/N); immune (anti-S/s)	Rare (anti-M/N); yes (anti-S/s)	Rare (anti-M/N); yes (anti-S/s)
P	Glycolipid	Unknown	Immune (anti-P); naturally occurring (anti-P ₁)	Yes (anti-P); rare (anti-P ₁)	Yes, mild

and MNSs systems (Table 13-1). These systems are defined by protein (as opposed to carbohydrate) antigenic determinants and, in general, antibodies to these antigens are acquired only after exposure by transfusion, pregnancy, or via HSCT. Some patients appear predisposed to develop antibodies and may form several antibodies simultaneously, which can limit the availability of donor blood. In the acute phase of alloimmunization to nonself protein antigens, T-cell-independent IgM antibodies may appear first, which subsequently isotype switch to IgG. As is the case with antibodies directed against antigens of the Rh blood group system, antibodies directed against other protein antigen systems are typically of the IgG isotype when discovered during pretransfusion testing.

Antibodies to certain blood group antigens are identified in patients more commonly than others, primarily due to differences in antigenicity and relative antigen frequencies in patient and donor populations. For example, the K antigen of the Kell blood group system is expressed on RBCs of approximately 10% of individuals of European ancestry. The remaining 90% of individuals are capable of mounting an immune response to K with a reasonable chance (10%) of receiving a unit of K⁺ cells if transfused. Consequently, anti-K antibodies are commonly identified antibodies.

Anti-K is the most common RBC antibody outside of the ABO and Rh systems. Anti-K can cause clinically significant hemolytic transfusion reactions (HTRs) and HDFN. Compared to Rh HDFN, HDFN due to anti-K

appears to have a lower degree of hemolysis and hyperbilirubinemia. The pathogenesis may be secondary to expression of Kell antigens at an earlier stage of erythropoiesis than Rh antigens. Anti-K may clear K⁺ erythroid progenitors at an early stage of development by fetal liver macrophages. Clearance of erythroid progenitors can lead to profound anemia, but with less evidence of hemolysis. Maternal anti-K antibody titers are a less reliable indicator of fetal risk than titers in Rh antibody-associated disease.

Among Kell antigens, K has a prevalence of 10% in individuals of European descent, 1.5% in Africans, and is rare in Asians. The k antigen is prevalent in all populations (>99%). Antibodies against 2 other Kell antigens, Kp^a and Js^a, are sometimes identified. Kp^a is a low prevalence antigen in most populations (<2%), while Js^a is rare in Europeans but relatively common (20%) in Africans. Weakened expression of all Kell antigens is associated with a rare phenotype—the McLeod phenotype that results from a deficiency of the Kx protein. The McCleod phenotype has been associated with several mutations and deletions at the XK locus that lies in close proximity to deletions associated with chronic granulomatous disease on the X chromosome. Individuals with McLeod phenotype have RBCs that are acanthocytic with decreased deformability and reduced survival, leading to a chronic but often well-compensated hemolytic anemia.

The Kidd blood group system is located on the erythrocyte urea transporter. Antibodies directed against antigens in the Kidd system are notorious for their involvement in

delayed hemolytic transfusion reactions (DHTRs). An individual is sensitized via transfusion, but the antibody titer decreases over time and becomes undetectable by standard serologic techniques at the time that the antibody screen is performed. The patient is then transfused with an ABO- and RhD-compatible unit and upon reexposure to the Kidd antigen, develops a rapid anamnestic antibody response that results in clinically significant hemolysis several days after the transfusion. The severity of DHTRs is compounded by the fact that Kidd antibodies, although IgG, fix complement and result in clinically significant intravascular hemolysis as well.

The Duffy antigens are structurally related to chemokine receptors that bind interleukin (IL)-8, monocyte chemotactic protein-1, and other chemokines, although its function on RBCs is not clear. It may allow RBCs to scavenge excess chemokines from the circulation. The Duffy glycoprotein also serves as a receptor for the malarial parasite *Plasmodium vivax*, which by selection pressure resulted in higher Fy(a⁻b⁻) frequency in individuals of African background where malaria is endemic. There is some evidence that the Duffy glycoprotein is expressed on nonerythroid tissue and represents a minor histocompatibility antigen in kidney transplantation. Alloantibodies against Duffy antigens may cause mild to severe acute or delayed hemolytic transfusion reactions and HDFN.

The MNSs blood group system is highly complex and includes 46 antigens that reside on one or both of the major RBC membrane glycoproteins—glycophorin A and glycophorin B. The RBC antigens M and N reside on glycophorin A, and alloantibodies to these antigens are usually IgM antibodies that are not reactive at 37°C and rarely are clinically significant. In contrast, alloantibodies to the S and s antigens, which reside on glycophorin B, are clinically significant IgG antibodies that can cause hemolytic transfusion reactions and HDFN. U is a high prevalence antigen, present in ~99.9% of individuals. Anti-U is difficult to manage because of the scarcity of antigen negative blood.

Other carbohydrate antigen blood group systems

Carbohydrate antigen systems other than the ABO system are rarely significant in clinical transfusion practice but are of interest for their role in specific infections and diseases. These systems include Lewis, P, and Ii.

Lewis antigens are technically not blood group antigens because they are not intrinsic to RBCs, but rather they are acquired passively by absorption from the plasma. The primary source of Lewis glycolipid in plasma is the gastrointestinal tract, where they are receptors for *Helicobacter pylori*. The 2 main antigens are Le^a and Le^b. Antibodies against Lewis antigens are typically IgM isotype

and occur naturally, so may be identified on routine antibody screens. In general, Lewis antibodies are not considered clinically significant and it is not necessary to transfuse antigen-negative RBCs.

RBCs are rich in P antigen, and include P₁, P₂, and P^k. Rare individuals who lack all P system antigens (pp phenotype) may produce a clinically significant antibody directed against the P antigen. These individuals also are resistant to parvovirus B19 infection because the P antigen on RBCs acts as the receptor for this virus. The P^k antigen is a receptor for Shiga toxins and P^k expression may also modulate host resistance to HIV infection. An autoantibody with P specificity is present in patients with paroxysmal cold hemoglobinuria (PCH), which most commonly occurs in children following a viral illness.

The Ii antigens serve as a scaffold for the synthesis of ABO antigens, and exhibit age-dependent expression patterns. In newborns, the predominant allele is the i antigen, which includes linear repeats of N-acetylglucosamine and galactose (N-acetylgalactosamine). After infancy, the predominant allele is the I antigen, which includes the same polysaccharides but is arrayed in a branched configuration rather than a linear configuration. Activity of the “branching enzyme” that forms the branched structure is absent in fetal erythrocytes but appears at about 6 months of age. Fetal and cord blood cells thus express strong i and weak I antigens, whereas adult RBCs express i weakly and I strongly. Individuals with infectious mononucleosis sometimes develop cold agglutinins directed against the i antigen, whereas people with *Mycoplasma pneumoniae* infections sometimes develop cold agglutinins directed against the I antigen. The I antigen is also the predominant specificity for RBC autoantibodies responsible for IgM-mediated autoimmune hemolytic anemia (AIHA) or cold agglutinin disease.

Similar to antibodies directed against ABO antigens, antibodies directed against other carbohydrate antigens are also usually IgM. One exception to this rule is with PCH, in which Donath-Landsteiner antibodies are cold-reacting IgG autoantibodies directed against the P antigen, can fix complement on circulating RBCs, and result in intravascular hemolysis. Of note, the DAT (direct antiglobulin or Coombs test) is usually paradoxically positive for complement and negative for IgG in PCH because the Donath-Landsteiner IgG autoantibodies usually detach from circulating RBCs after fixing complement. PCH is now most often associated with nonspecific childhood viral infections, but historically was associated with syphilis in adults.

Blood group genotyping

The molecular basis for most blood group antigens has been determined and demonstrates tremendous genetic

diversity, particularly in the ABO and Rh systems. The majority of blood group polymorphisms are caused by single-nucleotide polymorphisms in genes encoding protein antigens or genes encoding glycosyltransferases for the carbohydrate antigens. Many methods for RBC genotyping exist and vary in their complexity. Several blood group genotyping tests have been developed for the common antigens, but only one platform is currently approved by the Food and Drug Administration (FDA). These platforms typically exclude ABO and RHD given their allelic complexity. In the ABO system, more than 100 alleles encode the glycosyltransferases responsible for the ABO type. Genotyping methods have been developed to decrease the risk for erroneous ABO prediction but are unlikely to replace ABO typing by hemagglutination, which is extremely reliable, inexpensive, and has a quick turnaround time. Prototype *RHD* and *RHCE* platforms to test for multiple *RH* variants have been developed, but each of these targets many, but not all, known alleles.

The initial application of blood group genotyping was in the prenatal management of iso-immunized pregnancies. Fetal DNA extracted from amniocytes allowed for the determination of fetal RhD status in a mother known to be sensitized to RhD while avoiding the much riskier procedure of cordocentesis. The technology has evolved to permit analysis of free fetal DNA in maternal plasma, eliminating the risk of amniocentesis to test fetal RhD status. In some European countries, pregnant women who are Rh negative (with a partner who is Rh positive) and are not known to be sensitized, undergo such noninvasive molecular testing to determine fetal RhD status, which then dictates whether Rh immune globulin prophylaxis is given at 28 weeks gestation. In North America, such testing is less widely available and universal prenatal prophylaxis with Rh immune globulin in Rh-negative women remains the standard of care.

Genotyping can be used to determine RBC antigen phenotypes in patients recently transfused or with interfering allo- or autoantibodies, to resolve discrepant serologic typing, and/or when typing antisera are not readily available. Patients with warm autoantibodies whose RBCs are coated with IgG are difficult to antigen type with conventional antisera, and thus genotyping is an alternative to obtain the extended RBC antigen profile. Molecular typing can also facilitate complex antibody evaluations and guide RBC selection for patients with AIHA, SCD and thalassemia. Patients with SCD, who most often are of African background, have a high prevalence of *RHD* and *RHCE* variants, which can lead to Rh alloimmunization despite the provision of phenotypically Rh matched blood. High resolution *RH* genotyping can identify vari-

ant *RHD* and *RHCE*, which aids antibody evaluation and donor selection for future transfusion.

RBC genotyping is also an efficient method for donor centers to identify RBC units with rare or uncommon antigen phenotypes, or simply to meet demands for antigen-negative units. While identification of these donor units has historically been done serologically, automated DNA-based antigen testing can potentially improve the efficiency, reliability, and extent of matching.

Collection and storage of RBCs

CLINICAL CASE



A 29-year-old man with chronic renal failure has a hemoglobin (Hb) level of 6.7 g/dL and is seen in the emergency department for a shoulder injury. The patient has a normal heart rate and blood pressure. He states that he usually has this degree of anemia, has recently begun therapy with darbepoietin and iron through his nephrologist, and is able to conduct his daily routines without difficulty. The attending emergency department physician orders a blood transfusion.

Most RBCs collected in the United States are obtained from healthy volunteer donors. Collection of autologous RBCs and units from directed donors is possible but contributes only a small fraction of all RBC units collected. Whole blood collected from volunteer donors is fractionated into 1 or more transfusible components, including RBCs, platelets, plasma, and cryoprecipitate.

RBCs can be stored in anticoagulated plasma with or without additive solutions (AS). There are differences in the chemical composition of anticoagulant solutions and AS, but clinically they are generally used interchangeably. One commonly used AS for RBC storage is AS-1, which contains glucose, adenine, and mannitol. RBCs are stored routinely for up to 42 days at 4°C in currently available storage media. Techniques for freezing RBCs allow a shelf life of 10 years or greater and are used to cryopreserve RBC units with rare antigen combinations.

Cold storage of RBCs at 4°C has long been known to induce biochemical changes, such as decreased 2,3-diphosphoglycerate (2,3-DPG) levels, which are mostly reversible in vivo after transfusion. Retrospective studies in surgical patients suggested that transfusion of RBCs stored for >2 weeks was associated with increased postoperative complications and mortality. These and studies in other populations sparked several randomized controlled trials (RCTs) to prospectively investigate differences in outcomes

after the transfusion of fresher vs older stored RBCs in cardiac surgery (the RECESS trial), in intensive care unit (ICU) patients with respiratory failure (the ABLE and TRANSFUSE trials), preterm neonates (the ARIPI trial), and all hospitalized patients (INFORM-P). The results of these trials have not shown any clinical benefit in using “fresher” RBCs (generally <10 days) when compared to “older,” standard-issue RBCs.

Clinical transfusion of RBCs

Clinically, the starting point for transfusion is deciding whether it is indicated (further discussion below) and whether the patient has consented to transfusion. The next most important consideration for ensuring safe administration of blood products is definitive identification of the patient. Specifically, it is imperative that the labeling of the type and crossmatch sample, as well as the definitive identification of the unit to be transfused, occur at the patient’s bedside.

Selection of an RBC unit includes typing the patient’s RBCs for A, B, and D antigens; an antibody screen of the patient’s serum for antibodies to clinically significant RBC antigens; and performing a crossmatch, in which immunologic compatibility between the patient and the prospective RBC unit is assessed (see “Pretransfusion Testing”). Finding crossmatch-compatible blood for individuals who have been alloimmunized from prior pregnancies or transfusion, such as multitransfused patients with SCD, may take hours to days. Additionally, these patients require manual crossmatches, which take a minimum of 30 minutes to perform. Close communication with the blood bank about anticipated need for transfusion is critical. Accessing rare blood types through major regional or national blood centers that maintain collections of frozen RBC units may be required.

Table 13-2 summarizes the most commonly available RBC products and their respective indications. For acute blood loss, RBCs are used either alone or in combination with crystalloid and/or colloid solutions or plasma. Washed RBCs are indicated for patients who have had severe allergic or anaphylactic reactions to blood transfusion. Washed RBCs are rarely indicated to reduce the extracellular potassium load for adult patients, even those with renal insufficiency, but may be indicated if large volumes of older RBCs are transfused to an infant or neonate. Cryopreserved RBCs are primarily used for multiply alloimmunized patients who require units of rare RBCs.

Cellular blood products, including RBCs and platelets, are contaminated with small numbers of leukocytes sometimes referred to as passenger leukocytes. Passenger leukocytes play an important role in alloimmunization to

human leukocyte antigens (HLAs), transmission of cytomegalovirus (CMV) infection, cytokine-mediated febrile nonhemolytic transfusion reactions, transfusion-associated graft-versus-host disease (TA-GVHD), and other adverse events. Reduction in the number of passenger leukocytes (leukoreduction) results in clinically important reductions in the incidence of platelet transfusion refractoriness, alloimmunization to HLA antigens, and transfusion-transmitted CMV infection. As a result, there has been a trend toward the universal use of prestorage leukoreduction of both RBCs and platelets, particularly in patients who are likely to require prolonged transfusion support. Leukoreduction alone does not provide protection against TA-GVHD, so irradiation of all cellular blood products, in addition to leukoreduction, is necessary for patients at increased risk of TA-GVHD (see discussion in “Transfusion-associated graft-versus-host disease” below).

The primary goal of RBC transfusion is to improve the oxygen-carrying and delivery capacity of blood in patients with anemia. RBC transfusion can also aid in the overall management of hypovolemia in patients with intravascular volume depletion because of massive acute blood loss. Numerous compensatory mechanisms exist to maintain oxygen delivery in the face of anemia. These include increased heart rate and cardiac contractility, peripheral vasodilatation, increased oxygen delivery to tissues resulting from decreased oxygen affinity of hemoglobin due to increased erythrocyte 2,3-DPG concentration and decreased plasma pH, and altered oxygen consumption and utilization within the tissues. Studies in healthy people indicate that a shift to anaerobic metabolism occurs at hemoglobin levels of approximately 7.5 g/dL or lower when the blood hemoglobin concentration is reduced rapidly. Below this level, compensatory mechanisms to enhance oxygen transport are likely to be inadequate in patients with relatively rapid-onset anemia. Studies of Jehovah’s Witness patients, who refuse allogeneic blood transfusion, show that mortality increases with hemoglobin levels under 6 g/dL. However, there is no fixed hemoglobin target for RBC transfusion. An otherwise healthy individual may tolerate a blood hemoglobin concentration of 6 g/dL or less, especially if the anemia was gradual in onset. In contrast, those with severe cardiac or pulmonary disease or an individual with acute blood loss may require a higher blood hemoglobin concentration to maintain clinical stability.

A number of RCTs have been conducted to compare the effects of RBC transfusion with a restrictive policy (hemoglobin <7 to 8 g/dL) or a liberal transfusion threshold (hemoglobin <9 to 10 g/dL). Although these RCTs have been conducted in a variety of clinical settings, there

Table 13-2 Characteristics and indications for various RBC and platelet products

Product	Characteristics	Indication(s)
Whole blood	450 mL; coagulation factors adequate; platelets low in number; not widely available	To provide increased oxygen-carrying capacity and blood volume
RBCs	250–300 mL; can be stored up to 42 days	To provide increased oxygen-carrying capacity
Leukocyte-reduced RBCs	Contain $<5 \times 10^6$ leukocytes per unit	To reduce the incidence of febrile nonhemolytic reactions, CMV transmission, HLA alloimmunization, and platelet transfusion refractoriness
Leukocyte-reduced, irradiated RBCs	Leukoreduced and irradiated	To reduce the risk of transfusion-associated graft-versus-host disease, in addition to the benefits of leukoreduction listed above
Washed RBCs	Saline-suspended RBCs, 200–250 mL	To provide RBC support to patients with severe or recurrent allergic or anaphylactic reactions, patients with IgA deficiency with allergic reactions, and for intrauterine transfusions
Deglycerolized frozen RBCs	200 mL; rare RBCs are frozen in glycerol (to prevent hemolysis) and need to be washed and deglycerolized prior to transfusion	To support alloimmunized patients requiring RBCs with rare antigen combinations
Pooled platelets*	300–325 mL, 4–6 whole-blood donors	Prophylaxis and treatment of bleeding in the setting of thrombocytopenia or platelet dysfunction
Single-donor apheresis platelets*	150–350 mL, 1 apheresis donor	Same as pooled platelets; limits donor exposure
HLA-matched platelets*	Apheresis platelet from a donor with known HLA type, matched to patient	Immune-mediated platelet transfusion refractoriness with documented anti-HLA antibodies

*Platelet products should be subjected to leukoreduction or irradiation for the same indications as discussed for red blood cells.

are limitations that should be considered when applying to routine practice (ie, criteria for exclusion from study). The Transfusion Requirements in Septic Shock (TRISS) trial compared found that a restrictive transfusion threshold did not increase the risk of 90-day mortality or other adverse clinical outcomes. Evidence from the Transfusion Requirements in Critical Care (TRICC) trial indicates that hemodynamically stable patients in the ICU should not be transfused unless their hemoglobin is <7 g/dL. A 2013 RCT showed that a restrictive transfusion strategy improved outcomes in selected patients with acute upper-gastrointestinal bleeding. The Functional Outcomes in Cardiovascular patients Undergoing Surgical hip fracture repair (FOCUS) trial compared hemoglobin triggers of 8 g/dL vs. 10 g/dL in high-risk patients after hip surgery. The results of the FOCUS trial indicated that the restrictive strategy did not change functional outcome (walking across the room), nor did it result in an increase in cardiac events, even though the mean age was 82 years and a majority of patients had a history of cardiovascular disease. The Transfusion Requirements after Cardiac Sur-

gery (TRACS) trial established that a restrictive transfusion strategy (8 g/dL transfusion trigger) is noninferior to a liberal (10 g/dL transfusion trigger) strategy after cardiac surgery. The Transfusion Indication Threshold Reduction trial (TITRe2) also established equivalence of infectious and ischemic outcomes when comparing 7.5 to 9 g/dL transfusion triggers (35.1% vs 33.0%, respectively; $P=0.3$). Overall mortality was higher in the restrictive group (4.2% vs 2.6%), although 30-day mortality was similar (2.6% vs 1.9% for restrictive and liberal arms, respectively). The evidence regarding a restrictive or liberal transfusion strategy in patients with acute coronary syndromes is unclear.

In the clinical case previously described, the attending physician's initial decision to administer RBCs in response to a hemoglobin value, without taking the patient's overall presentation into account, failed to consider this individual with gradual-onset anemia could tolerate the low hemoglobin level without significant difficulty. The case illustrates the importance of using clinical judgment in making transfusion decisions rather than arbitrary hemoglobin cutoffs.

KEY POINTS

- The ABO system is the most important determinant of transfusion compatibility.
- Rh compatibility is necessary because of the high immunogenicity of the RhD antigen and the role of anti-D antibodies in HDFN and delayed hemolytic transfusion reactions.
- Other frequently relevant blood group systems include Kell, Kidd, Duffy, and MNSS.
- There is no fixed threshold for transfusion of RBCs. RCT evidence to date supports restrictive RBC transfusion strategies in many clinical settings.

Platelet transfusion

The ABO system

Platelets express A, B, and H antigens to varying degrees. Unlike RBC or plasma transfusion, ABO compatibility does not necessarily need to be honored for platelet transfusion. About 10% of group A and B individuals have high antigen expression, which can impact platelet increments in major ABO-incompatible transfusion (see “Choice of platelet product” below).

The HLA system

Alloimmunization to HLA antigens is the major cause of immune-mediated refractoriness to platelet transfusion in patients undergoing chronic platelet transfusion therapy. Overall, however, nonalloimmune causes of platelet refractoriness are significantly more common (eg, immune-mediated thrombocytopenia [ITP], hypersplenism, and consumptive coagulopathy).

Although only HLA class I antigens at the HLA-A and HLA-B loci have been shown to be important in causing immune-mediated refractoriness to platelet transfusion, given the high degree of polymorphism in the HLA system, large numbers of HLA-typed donors need to be available to blood centers to provide HLA-compatible platelets to individual patients. If HLA-matched platelet donors are not available, identification of the specificity of the patient’s HLA antibodies may allow blood centers to provide antigen-negative platelets for transfusion (ie, platelets that do not express HLA antigens against which the patient has known antibodies). Crossmatching platelets is another technique for finding compatible units. HLA antigens can be categorized into groups with common epitopes that may cross-react with the same HLA antibodies; these groups of HLA antigens are referred to as cross-reactive groups. When an exact HLA-identical

platelet donor is not available, blood centers can use cross-reactive groups to locate platelet donors in whom the risk of cross-reactivity between the recipient’s antibodies and the donor’s antigens may be minimized.

Human platelet antigens

In addition to anti-HLA antibodies, antibodies to platelet-specific antigens may also cause platelet transfusion refractoriness. The human platelet antigens (HPAs) arise as a result of polymorphisms involving various platelet membrane glycoproteins. Differences in HPA allelic frequencies in different ethnic populations may partially account for differences in the rates of alloimmunization to HPA antigens reported by different investigators.

There are a number of well-characterized HPA antigen systems, but alloimmunization is most commonly due to polymorphisms involving the HPA-1a/1b system (previously known as the PLA1/A2 system). The HPA-1a/1b system arises from a polymorphism on the β_3 subunit of the platelet fibrinogen receptor, GPIIb/IIIa, also known as integrin $\alpha_{2b}\beta_3$ or CD41/CD61. In addition to ethnic differences in allelic frequencies, alloimmunization to HPA-1a is strongly associated with expression of HLA-DRB3*0101 and HLADQB1*0201 in the recipient.

Alloimmunization to HPAs can cause neonatal alloimmune thrombocytopenia (NAIT) and posttransfusion purpura (PTP) and accounts for a small proportion of immune-mediated platelet transfusion refractoriness in multiply transfused platelet transfusion recipients. There are case reports of alloimmune thrombocytopenia after HPA-mismatched allogeneic HSCT. PTP occurs when transfused platelets are destroyed by HPA alloantibodies through a process analogous to a delayed hemolytic transfusion reaction. However, following exposure to the HPA antigen in question through RBC or platelet transfusion, what then follows is the apparent immune destruction of the patient’s own antigen-negative platelets, in addition to any transfused antigen-positive platelets. The mechanism by which autologous platelets are destroyed in PTP is unclear, although cross-reactivity of HPA alloantibodies to patient platelets is a favored explanation. For the patient with a history of PTP, RBC units should be washed to remove any contaminating platelets that could incite an additional episode of PTP. For platelet transfusions, alloantigen-negative platelets should be selected.

Collection and storage of platelets

Two types of platelet products are routinely available for clinical use: pooled and single-donor platelet (SDP) products. Pooled platelets are obtained by pooling individual platelet concentrates derived from whole blood units

obtained from 4 to 6 volunteer, ABO-identical whole-blood donors. The platelet content of pooled platelet products varies depending on the number of units in the pool and various technical factors.

SDPs are collected from single donors using continuous centrifugation plateletpheresis techniques in which RBCs and plasma are returned to the donor. Plateletpheresis collection techniques have been refined such that a minimum of 3×10^{11} platelets—that is, approximately the same number of platelets contained in a pool of 6 whole blood-derived platelets—can be collected from a single donor in a single session.

As with RBCs, leukoreduction of platelet products can reduce the incidence of platelet transfusion refractoriness, alloimmunization to HLA antigens, transfusion-transmitted CMV infection, and febrile nonhemolytic transfusion reactions. For optimal viability and function, platelets must be stored at room temperature, which increases the risk of bacterial growth and currently limits the storage of platelets to 5 days. Clinical studies indicate that there is relatively little loss of platelet function and viability during this time. The storage lesion primarily involves platelet activation, which is reflected in platelet shape change, adhesion, aggregation, secretion of platelet granular contents, and the expression of activation antigens.

In 2015, the FDA approved cold-stored platelets, which are stored at 4°C without agitation for 3 days. Cold-stored platelets are used only for resuscitation in actively bleeding patients. Refrigeration activates platelets (eg, increased P-selectin expression) and renders them more immediately efficacious after transfusion, even if posttransfusion platelet increments are lower than with room temperature–stored platelets.

After routine platelet collection, 2 additional modifications to platelet products are available: platelet additive solution platelets and pathogen-reduced platelets. Platelet additive solution platelets provide a metabolically optimized environment for storing platelets and also reduce the plasma content of platelet components to minimize transfusion reactions. With less plasma in the component, a lower incidence of allergic transfusion reactions has been demonstrated, and there is a theoretical reduction in risk of transfusion-related acute lung injury (TRALI). Pathogen reduction is achieved by treatment of the platelet product with amotosalen (psoralen)/UV, riboflavin/UV, or UV alone. In the United States, only amotosalen is FDA approved. A primary benefit of pathogen-reduced platelets is a significant decrease in bacterial contamination, which is the primary infectious complication of platelet transfusion. This method also reduces the plasma content in the platelet product, which reduces the incidence of

transfusion reactions. Pathogen-reduced platelets using riboflavin and UVB inactivation steps have been shown in a randomized trial to be noninferior to standard platelets in terms of World Health Organization (WHO) bleeding outcome; however, platelet increments after transfusion are ~50% lower with pathogen inactivation.

Clinical transfusion of platelets

CLINICAL CASE



A 56-year-old multiparous female develops acute myeloid leukemia and receives induction therapy. Her platelet count decreases to $<10,000/\mu\text{L}$. The patient initially responds well to prophylactic transfusion with pooled platelet concentrates. Later in the hospitalization, her 1-hour posttransfusion platelet count increments are persistently $<5,000/\mu\text{L}$. Having obtained HLA typing on the patient before induction, the attending physician asks the blood bank for HLA-matched platelets.

Prophylactic platelet transfusion

Bleeding in thrombocytopenic patients occurs at all platelet counts, but several studies indicate that the rate of spontaneous bleeding does not dramatically increase until the platelet count is $\leq 5,000/\mu\text{L}$. Several prospective RCTs show no differences in hemorrhagic risks between prophylactic platelet transfusion triggers of $\leq 10,000$ and $\leq 20,000/\mu\text{L}$. A randomized, controlled noninferiority trial of no-platelet prophylaxis vs prophylaxis (TOPPS trial) in patients with hematologic malignancies demonstrated no prophylaxis was statistically inferior to prophylaxis, with a trigger of $<10,000/\mu\text{L}$, although not by a large margin: WHO grade 2 or higher bleeding was 50% with no prophylaxis vs 43% with prophylaxis.

Indications for raising the prophylactic platelet transfusion target include blast crisis or acute promyelocytic leukemia during induction; recent or imminent invasive procedures; qualitative platelet dysfunction due to uremia, drugs, or genetic defects; concurrent coagulopathy; fever; hypertension; and acute pulmonary processes. In patients with significant active bleeding, most clinicians target the platelet count to 50,000 or up to 100,000/ μL in patients with definite or suspected central nervous system bleeding. Realistic target counts should be set in patients who do have inadequate posttransfusion increments, such as those with splenomegaly or immune-mediated platelet transfusion refractoriness. Checking an immediate (10 to 60 minutes) postinfusion platelet count is a necessary screen for platelet refractoriness. Table 13-2

summarizes the major platelet preparations and their respective indications.

Choice of platelet product

The current evidence indicates that apheresis platelets and pooled platelets can generally be used interchangeably for most platelet transfusions. Alloimmunization rates, acute reaction rates, and transfusion-related acute lung injury rates are not meaningfully different. An argument that often has been proposed in favor of apheresis platelets over pooled platelets is the theoretical reduction in the incidence of transfusion-transmitted infectious diseases. Given the very low absolute magnitude of the infectious risk associated with transfusion of blood products (discussed in the section “Infectious complications” later in this chapter), the cost effectiveness of requiring single-donor transfusions for all platelet transfusion recipients is questionable.

In contrast to the availability of universal RBC donors (blood group O negative) and universal plasma donors (blood group AB), universal donors for platelets do not exist because platelet products contain both platelets and a substantial quantity of plasma (typically ~300 mL). For example, group O platelets contain anti-A and anti-B isoantibodies that would react against the RBCs of all but type O recipients. Indeed, clinically apparent hemolysis is occasionally observed after minor (plasma) incompatible platelet transfusion; rarely, hemolysis is severe. Major (cell) incompatible transfusion (eg, A platelets transfused into an O recipient) may yield up to 20% lower posttransfusion increment because the recipient’s isoantibodies result in immune-mediated clearance of platelets expressing the incompatible ABH antigens. Ideally, patients should receive ABO-identical platelets; in reality, platelets are in short supply and sometimes chosen for other characteristics (eg, HLA-matched), so ABO matching is frequently not followed. Blood banks have varying procedures and policies for selection of type-specific platelet product transfusions.

Platelet products are selected for RhD compatibility. Transfusion of a platelet product from an RhD-positive donor to an RhD-negative recipient uncommonly (<1% incidence) may result in anti-D antibody formation because of exposure to the minimal volume of residual RhD positive RBCs in the platelet product. In situations in which Rh negative platelets are unavailable and platelet transfusion is required, Rh immune globulin (RhIG) may be used to prevent alloimmunization to RhD, particularly in females of childbearing potential.

Platelet transfusion dose

The dose of platelets administered to a thrombocytopenic patient depends on the therapeutic goal. If the primary

goal is to administer a sufficient number of platelets to prevent bleeding in an uncomplicated patient, the target typically would be to transfuse when the platelet count drops below 10,000/ μ L. The appropriate platelet dose depends on many factors—including the size of the patient and the presence of splenomegaly, active bleeding, platelet consumption (eg, DIC), anti-HLA or other antiplatelet antibodies, and the overall clinical scenario.

FDA standards dictate that single-donor apheresis platelets must contain at least 3×10^{11} platelets and that individual platelet concentrates prepared from single units of whole blood must contain at least 5.5×10^{10} platelets; that is, the equivalent of approximately 3×10^{11} platelets per 5- or 6-pool. In an average-size patient, in the absence of any of the risk factors for poor platelet transfusion response listed previously, approximately 3×10^{11} platelets is considered an appropriate adult dose, and it is expected to increase the platelet count by 20,000 to 50,000/ μ L. If a patient is being managed as an outpatient, larger doses of platelets may extend the interval between transfusions. A multicenter RCT (Platelet Dose [PLADO] Trial) compared low-, typical-, and high-platelet doses of prophylactic platelets for a platelet count of 10,000/ μ L in patients undergoing chemotherapy or HSCT. WHO grade 2 or higher bleeding was the same in all groups, and the low dose group (1.1×10^{11} platelets—half of a standard dose) received significantly fewer platelets, albeit over more transfusion episodes.

Diagnosis and management of platelet transfusion refractoriness

A commonly used bedside definition of platelet transfusion refractoriness is 2 consecutive postinfusion platelet count increments $\leq 10,000/\mu$ L. A more formal definition of refractoriness, which adjusts for both the size of the patient and the number of platelets actually infused, uses the corrected count increment (CCI), which is based on a platelet count obtained within 1 hour of transfusion, calculated as follows: CCI = body surface area (BSA; m^2) \times platelet count increment $\times 10^{11}$ / number of platelets transfused. For example, if 3×10^{11} platelets (standard dose, as described above) are transfused to a patient with a BSA of $1.8 m^2$, and the posttransfusion increase in platelet count is $23,000/\mu$ L, then the CCI = $1.8 m^2 \times 23,000/\mu$ L $\times 10^{11} / 3 \times 10^{11} = 13,800$. Platelet transfusion refractoriness often is defined as 2 or more consecutive postinfusion CCIs of $< 5,000$ to $7,500$.

A trial of fresh, ABO-matched platelets may increase the posttransfusion increment modestly. A majority of platelet refractoriness is caused by nonimmune pathophysiological conditions that consume platelets regardless of the platelet product transfused (eg, splenomegaly, DIC,

fever, hemorrhage). Among immune-mediated causes, alloimmunization to HLA antigens accounts for most cases of platelet transfusion refractoriness; rarely, HPA incompatibility is responsible. In the absence of obvious non-immune causes of platelet transfusion refractoriness, an anti-HLA antibody evaluation is warranted. Anti-HLA antibodies and their specificities are detected on high-throughput platforms such as Luminex microbeads coated with HLA class I and II antigens. In patients whose panel-reactive antibody screen is positive (positive is defined by each lab), platelets should be selected based on HLA matching, avoiding the antibody specificities found in the patient, or platelet crossmatching, although these methods do not guarantee improved platelet responses. There is no evidence that the use of single-donor or HLA-matched platelets enhances response to platelets in the absence of documented alloimmunization to HLA antigens. Alloimmunization sometimes resolves spontaneously; thus, the requirement for HLA-matched products may not persist indefinitely.

Platelets express all HLA class I antigens, but HLA-A and HLA-B antigens are the clinically significant antigens in immune platelet refractoriness. Therefore, most blood centers optimize matching only at the HLA-A and HLA-B loci. The HLA type of an individual determines the difficulty in locating platelets that are reasonably HLA compatible. Grading systems can semiquantitatively define the degree to which the platelet donor and the platelet recipient are matched at these loci, although the predictive values are modest.

The relatively low-stringency, serologic, 4-loci, HLA-matching protocols typically used to select platelet products is quite different from the relatively high-stringency, molecular-level, 10- to 12-loci, HLA-matching used to select HSCT donors. Nevertheless, for some patients with unusual HLA types locating an appropriate HLA-matched platelet donor may still be difficult and relying solely on HLA matching has certain shortcomings. For these reasons, platelet crossmatching is an alternative approach, similar to that used in RBC compatibility testing: a sample of the patient's serum is incubated with aliquots of platelets from candidate donor units, and those units that manifest the least cross-reactivity are selected for transfusion. It is not clear which method (HLA matching or platelet crossmatching) is superior, and some centers use a combination of both methods. Even when a suitable HLA-matched donor is identified, it can take several days to obtain a product for transfusion, as the donor typically has to be called in to donate specifically for the patient in question, and the subsequent donation must undergo all infectious disease testing before release.

A variety of approaches have been taken when no compatible platelets can be found for a patient who is alloimmunized to HLA antigens. Platelet transfusion refractoriness in HSCT recipients can be managed by obtaining platelets from the original stem cell donor. In other settings, therapeutic modalities include corticosteroids, plasmapheresis, intravenous immunoglobulin (IVIg), frequent platelet transfusion, continuous-infusion platelet transfusion, and aminocaproic acid. Clinical data do not clearly support any one of these modalities over the others. Realistic targets and infusion schedules should be set in alloimmunized patients who are not responding well to platelet transfusion or those for whom HLA-matched products are unavailable. Transfusion of multiple units of platelets from random donors, whether pooled or apheresis, with no realistic expectation of an increase in platelet count or cessation of bleeding, exposes the patient to all the risks of transfusion with no benefit. In addition, it may result in reduced availability of platelet products for other patients when supplies are limited.

KEY POINTS

- Platelets express ABH and HLA class I antigens, which can occasionally be clinically significant.
- Human platelet antigens are polymorphisms on platelet surface glycoproteins that may also mediate platelet transfusion refractoriness, as well as NAIT, PTP, and alloimmune thrombocytopenia following HSCT.
- Although non-immune mechanisms are the most common causes of platelet refractoriness, antibodies directed against HLA antigens can develop following blood transfusion or pregnancy and are the most important cause of immune-mediated platelet transfusion refractoriness.
- Prophylactic platelet transfusion should be considered when the peripheral blood platelet count decreases below 10,000/ μ L in uncomplicated patients. The platelet count target should be increased in the presence of additional risk factors for bleeding or platelet consumption.

Granulocyte transfusion

Granulocyte antigen systems

There are 5 human neutrophil antigen (HNA) systems that represent polymorphisms on a variety of neutrophil cell surface proteins, although expression of HNA-3 (CTL2), HNA-4 (CD11b), and HNA-5 (CD11a) is not restricted to neutrophils. HNA-1 ($Fc\gamma$ RIIb, CD16b) appears to be the most commonly antigenic. $Fc\gamma$ RIII is linked to the outer leaflet of the cell membrane bilayer by

a glycosylphosphatidylinositol (GPI) anchor. As a result, HNA-1 antigens are poorly expressed on neutrophils in patients with PNH as well as in a proportion of patients with a variety of other clonal myeloid disorders, including some patients with myeloid leukemia, in which the expression of GPI-linked proteins has been reported to be absent or reduced. Common properties of HNA systems include their absence on early myeloid precursors and the acquisition of expression during neutrophil differentiation. Donor-recipient or fetal-maternal mismatches involving the HNA antigens appear to be responsible for a significant percentage of reported cases of neonatal alloimmune neutropenia (NAIN), granulocyte transfusion refractoriness, TRALI (see “Transfusion-related acute lung injury” below), and delayed neutrophil recovery or secondary graft failure following HSCT.

HNA alloantibodies appear to play an important role in some cases of febrile nonhemolytic transfusion reactions and TRALI. In one study, more than one-third of patients undergoing HSCT acquired antibodies directed against neutrophils in the posttransplantation period; the presence of such antibodies was independently correlated with both delayed neutrophil engraftment and postengraftment neutropenia. The latter observation is important because such patients often respond to steroids or granulocyte colony-stimulating factor (G-CSF) and thus may be able to avoid retransplantation. In some patients alloimmunized to neutrophil-specific antigens, transfused granulocytes do not migrate to sites of infection, which suggests that some neutrophil-specific antibodies can interfere with qualitative neutrophil function.

Collection and storage of granulocytes

Approximately 10^{10} granulocytes can be harvested from a healthy donor during a single leukapheresis session. Pretreatment with corticosteroids induces neutrophilia in donors, increasing the granulocyte yield. Pretreatment of granulocyte donors with G-CSF significantly increases the granulocyte yield. Several studies suggest that administering G-CSF to healthy donors does not lead to an increased incidence of hematologic disorders. Because of the short half-life of granulocytes and 24-hour expiration time of the component, granulocytes should be harvested, transported, and infused into the intended recipient within hours. This conflicts with the time required for infectious disease screening of the donor, which can take 24 to 48 hours to complete. Consequently, some institutions have procedures in which a treating physician can authorize transfusion of the product before infectious disease testing has been completed. In these situations, the physician is given the opportunity to weigh the potential benefit of

granulocyte transfusions with the putative risk of infectious disease transmission by the blood product. Granulocyte donors are typically selected from regular apheresis platelet donors who have had documented negative infectious disease testing within the prior month, or the PBSC donor (in matched-related transplants) if applicable. Some blood centers also may bring the donor in on the day before the granulocyte donation to collect samples for infectious disease testing to ensure that results are available before release of the granulocyte product.

Clinical transfusion of granulocytes

Most cases of prolonged marrow aplasia can be treated adequately without granulocyte transfusion. The initial treatment of patients with neutropenic fever consists of broad-spectrum antibiotics and recombinant growth factors. Granulocyte transfusions should be considered only in patients with a realistic expectation of marrow recovery who have ongoing neutropenia with persistence or progression of bacterial or fungal infection despite appropriate antibiotic and antifungal therapy. Although underpowered, the RING trial on the efficacy of high-dose granulocyte transfusion therapy in neutropenic patients with infection did not show a benefit of granulocytes in neutropenic patients, as compared to conventional therapy.

Once the decision to use granulocyte transfusions has been made, an adequate dose should be given. A minimum dose of 2×10^{10} to 3×10^{10} neutrophils should be given to adults. Achieving this dose requires transfusing multiple units from unstimulated donors or using a collection method that increases the granulocyte yield from a single donor, such as pretreatment of the donor with corticosteroids or G-CSF. Because of the high volume of contaminating RBCs, ABO-compatible donors need to be used unless effective RBC sedimentation is performed. Granulocyte transfusions are continued, as they are available from donors, until the infection is controlled; until the patient's neutrophil count has increased to $>500/\mu\text{L}$; or until significant toxicity, particularly pulmonary toxicity, occurs. Patients with alloantibodies to granulocyte-specific antigens may not achieve a satisfactory therapeutic response to granulocyte transfusions and are at higher risk of pulmonary toxicity. Granulocyte transfusions should be separated temporally from amphotericin administration because case series evidence suggests that pulmonary toxicity otherwise is increased. Serologic testing for anti-neutrophil antibodies is not performed routinely, but it is indicated if significant transfusion reactions develop. If antibodies are found, leukocytes from compatible donors may be used. Leukocyte reduction filters obviously should not be used with granulocyte products. If the potential for

CMV transmission is a concern, then granulocytes collected from CMV-seronegative donors should be used. Unlike stem cells and donor lymphocyte infusions, however, granulocytes should undergo irradiation. Because granulocytes have a short lifespan, they must be transfused as soon as possible and within 24 hours of collection. In this time, it is usually not feasible to obtain transfusion-transmitted disease testing results. To mitigate infectious transmission risk, frequent donors who have recently tested negative are selected, and physicians must document that they consent to the risk of transfusing an unlabeled product.

KEY POINTS

- Antibodies directed against HNA system antigens can mediate TRALI, refractoriness to granulocyte transfusions, NAIN, alloimmune neutropenia following HSCT, febrile transfusion reactions, and qualitative neutrophil dysfunction.
- Transfusion of granulocytes can be considered in patients with severe prolonged neutropenia and antibiotic-refractory infections as a bridge to endogenous granulocyte recovery.

use of ultraviolet-activated psoralen derivatives. Psoralen or riboflavin-based UV treatment systems are new methods in the United States.

In theory, plasma could be used to treat acquired or congenital deficiencies of virtually any individual pro- or anticoagulant factor. It is standard practice, however, to use recombinant or purified pharmaceutical preparations of coagulation-related proteins when available and replacement of a single factor is indicated. Thus, the most common indications for plasma transfusion therapy include situations in which multiple factor deficiencies are present simultaneously, such as patients with liver disease, DIC, vitamin K deficiency (nutritional or due to warfarin therapy requiring urgent therapy), dilutional coagulopathy of massive transfusion secondary to acute blood loss, or plasma exchange for such indications as thrombotic thrombocytopenic purpura (TTP). ADAMTS13 (a disintegrin and metalloprotease with thrombospondin) protease activity is stable up to 5 days of thawed storage and, as such, thawed plasma can be used for plasma exchange in TTP. Four-factor prothrombin complex concentrates with adequate FVII content (in addition to prothrombin, FIX, and FX) increasingly are being used for urgent warfarin reversal in conjunction with vitamin K, particularly in the setting of intracranial hemorrhage.

Prophylactic plasma transfusions to correct mild prolongations of coagulation values before an invasive procedure usually are not indicated. RCTs to determine the appropriate indications and dosing of plasma therapy have not been completed, in part because of the low baseline bleeding risk associated with minor coagulopathies and invasive procedures, making appropriately powered trials prohibitively large. The transfusion reaction risks (eg, TRALI, allergic reactions, and fluid overload) often outweigh the speculated benefits of plasma transfusion. However, when clinically indicated, plasma is typically dosed at 10 to 20 mL/kg.

Cryoprecipitate

Cryoprecipitate is prepared by thawing FFP at 4°C and then removing the supernatant from the cryoprecipitable proteins following centrifugation at 1°C to 6°C. Cryoprecipitate is a concentrated preparation of procoagulant factors, including fibrinogen, factor VIII, VWF, factor XIII, and fibronectin. Although cryoprecipitate contains a subset of procoagulants, unlike plasma, it does not contain appreciable quantities of physiologic anticoagulants, such as protein C or protein S. Cryoprecipitate alone is not indicated in patients with disease processes that deplete both procoagulants and anticoagulants, such as DIC or severe hepatic failure. Historically, cryoprecipitate was used to treat

Transfusion of plasma products

Plasma

Units of plasma are usually obtained from volunteer whole blood units. The traditional nomenclature of fresh frozen plasma (FFP) applies to plasma frozen within 8 hours of collection and used within 24 hours of thawing. Other types of plasma commonly used interchangeably with FFP include plasma frozen within 24 hours of collection and used within 24 hours of thawing (PF24), and thawed plasma, which is made from FFP or PF24 and kept refrigerated for up to 5 days after thawing. These products often are used interchangeably; however, because of the decrease in levels of the heat labile factors V and VIII over time, thawed plasma should not be used as the sole source of factor replacement in patients who are significantly deficient in either of these factors. A standing inventory of thawed plasma is typically available quickly in emergency bleeding situations in large centers. New viral inactivation methods to reduce pathogens in plasma have recently been approved. The most common of these techniques uses a solvent detergent method that disrupts lipid-containing viruses. Methylene blue is another method of pathogen inactivation, commonly used in Europe, in addition to the

von Willebrand disease, hemophilia A, and congenital fibrinogen disorders, but now recombinant factors and virally inactivated factor concentrates are widely available. Cryoprecipitate has also been used to treat qualitative platelet dysfunction due to uremia and life-threatening hemorrhage secondary to thrombolytic therapy. The supernatant plasma (sometimes referred to as *cryosupernatant* or *cryo-poor plasma*), which lacks the high-molecular-weight multimers of VWF, can be used in the treatment of TTP but does not appear to be superior to plasma for this indication. Cryoprecipitate is not pathogen inactivated, and a pool of 8 to 10 units of cryoprecipitate is needed to correct hypofibrinogenemia in an adult, resulting in multiple donor exposures. For children, the appropriate dose is 1 unit of cryoprecipitate per 10 kg of body weight.

Immunoglobulin

Commercially available IVIg products are typically prepared by cold ethanol fractionation of large pools of human plasma followed by viral inactivation procedures, such as solvent detergent treatment or heat pasteurization. As is the case with virally inactivated plasma, the risk of transmission of hepatitis B virus (HBV), hepatitis C virus, or HIV appears to be negligible, although concerns remain regarding the potential transmission of certain difficult-to-inactivate pathogens, such as parvovirus B19 and prions. There have been reports of acute renal failure occurring in association with the administration of IVIg, particularly in patients with preexisting renal insufficiency, hypovolemia, diabetes, or other risk factors. Most of the immunoglobulin in commercially available preparations of IVIg is IgG itself, and the IgG immunoglobulin subtype distribution (ie, IgG₁ through IgG₄) is similar to that found in normal human plasma. Relatively small amounts of IgA and IgM also are present. IVIg has been used to treat a variety of hematologic disorders, including congenital immunodeficiency syndromes, ITP, autoimmune neutropenia, and recurrent bacterial infections occurring in association with chronic lymphocytic leukemia, multiple myeloma, and other immune dysregulation conditions. In autoimmune cytopenias such as ITP, IVIg is considered first-line intervention when a rapid response is required, although the effect may be transient. Preparations of subcutaneously administered immunoglobulin are also available.

The mechanism by which IVIg ameliorates autoantibody destruction of blood cells is not clearly elucidated. Historically, it was believed that the infused IgG blocks Fc receptors on phagocytic cells of the reticuloendothelial system, but other evidence supports IVIg glycosylation driving increased inhibitory IgG receptor expression, for-

mation of immune complexes that interact with activating dendritic cell Fc receptors, as well as direct T- and B-cell interactions.

A significant proportion of patients receiving IVIg develop a positive DAT because of the presence of anti-A or anti-B antibodies derived from type O individuals in the donor pools. Overt, acute alloimmune hemolytic anemia can also develop, especially for blood group A and AB recipients following multiple doses of IVIg given in close proximity. Fever is a relatively common sequela of IVIg administration and does not necessarily preclude the administration of additional IVIg.

KEY POINTS

- The most common indications for plasma transfusion include rapid reversal of warfarin effects; treatment of deficiencies of coagulation factors for which specific coagulation replacement products are not available; and plasma exchange in patients with TTP.
- The most common indication for transfusion of cryoprecipitate is hypofibrinogenemia in the context of complex coagulopathy (eg, DIC). Fibrinogen concentrates are available for selective fibrinogen replacement.

Pretransfusion testing

The term *pretransfusion testing* refers to the series of laboratory tests that blood banks and transfusion services perform to provide immunologically compatible blood products to patients. It is important for hematologists to have a general working knowledge of what takes place behind the scenes in the blood bank between the time when blood is ordered and when it is received.

ABO/Rh(D) typing

Determining a patient's ABO blood group includes 2 independent sets of tests that are expected to yield complementary results. In the *forward typing*, patient RBCs are mixed with IgM anti-A or anti-B reagent typing sera. Agglutination of cells with either reagent indicates the presence of the A or B antigen, respectively, on the patient's RBCs. Because of the importance of determining a patient's ABO blood type with absolute certainty, a second test known as *reverse typing* is performed for confirmation. Naturally occurring isohemagglutinins to A or B antigens occur in individuals whose RBCs lack those antigens. The patient's serum or plasma is mixed with reagent RBCs expressing either A or B antigens, and agglutination is assessed. Table 13-3 illustrates the expected forward- and reverse-typing results for the 4 possible ABO blood types.

Table 13-3 ABO blood group typing reaction results

Patient's ABO type	Forward typing		Reverse typing	
	Reaction of patient's RBCs with:	Reaction of patient's serum with:	A1 RBC	B RBC
	Anti-A	Anti-B		
O	0	0	+	+
A	+	0	0	+
B	0	+	+	0
AB	+	+	0	0

Discrepancies between forward- and reverse-typing reactions occur and can sometimes be explained by evaluating the patient's recent transfusion history. For example, a blood group B individual given type O red cells in an emergency situation could continue to demonstrate only the appropriate anti-A antibodies by reverse typing but show a mixed field of RBCs, that is, both agglutinated (the patient's blood group B cells) and unagglutinated (the transfused blood group O cells) RBCs upon forward typing with anti-B reagent typing sera. Forward- and reverse-typing discrepancies are expected in newborns because isoantibody production is delayed for several months while their immune systems mature. For this reason, only forward typing is performed on newborns. Forward and reverse ABO typing discrepancies also occur in patients who have undergone ABO-mismatched HSCTs, particularly during their transition from one blood type to another. Additionally, typing discrepancies can result from genetically distinct A and B blood group subtypes or rare acquired phenotypes. It is important to determine the etiology of ABO typing discrepancies to select the appropriate ABO type for different blood components.

Typing for the presence or absence of the Rh(D) antigen on RBCs is also an important part of determining a patient's blood type. Typing for D does not involve a reverse typing similar to ABO typing because anti-D is not normally expected to be present in the sera of Rh-negative individuals. Please refer to the section on Rh antigens for discussion of weak D vs partial D phenotypes and their clinical significance.

Antibody screen and specificity identification

In general, a patient who has never been pregnant or transfused is expected to have only the naturally occurring isoantibodies based on his or her ABO type. However, it is required to test all patient sera for the presence of RBC alloantibodies. If any clinically significant alloantibodies are detected, then ABO-compatible RBCs lacking the corresponding antigen(s) must be selected for transfusion.

Antibody screening consists of testing patient serum or plasma with 2 or 3 reagent RBCs whose extended phenotype has been characterized for all major common, clinically significant RBC antigens. If the patient's serum does not react with the screening cells, then ABO-compatible units can be selected for crossmatching. A negative antibody screen does not exclude all alloantibodies, only antibodies to the common, clinically significant antigens.

If the patient's antibody screen is positive, further testing is required to determine the specificity of the antibody (or antibodies) present. To accomplish this, the patient serum is tested against a larger set of reagent RBCs (typically 11 to 16, referred to as an RBC panel). By comparing the resulting pattern of reactivity (ie, which cells agglutinate and which do not) with the phenotype of each of the reacting and nonreacting reagent RBCs, alloantibody specificities can be identified. All reagent RBCs are blood group O, so that the presence of anti-A or anti-B isoantibodies does not affect the results. Based on results of the antibody identification panel, ABO/Rh-compatible RBC units are selected from inventory; RBCs from attached segments from each of the units are phenotyped using reagent antisera to identify antigen-negative units.

Several agglutination methods for antibody screening are available, including tube, gel-based and solid-phase testing. Differences in the sensitivity, specificity, and interfering substances in the detection of clinically insignificant antibodies exist among the available methods. The gel and solid phase methods are formulated specifically to identify IgG antibodies and not detect IgM antibodies. Therefore, if the purpose of testing is to evaluate for the presence of a cold agglutinin or other IgM antibody, consultation with a blood bank physician is important to ensure the appropriate test method is used. The antibody screen, indirect antiglobulin test, and indirect Coombs test are all different names for the same assay.

Antibody identification may take several hours to several days to complete, depending on the complexity of the reactivity. Patients with warm autoantibodies or AIHA present a significant challenge to transfusion services because of the presence of panreactive antibodies (ie, the antibodies bind to the patient's own RBCs but also to all other RBCs, including reagent screening and panel cells). As a result, the presence of additional alloantibodies to specific blood group antigens may be masked by the autoantibodies. Time-consuming absorption techniques must be used in these cases and are discussed in the section "Autoimmune hemolytic anemia" in this chapter.

Crossmatching

Two basic types of crossmatch procedures are used depending on results of the patient's antibody screen. If the

antibody screen is negative and the blood bank has historical records indicating no alloantibodies in the patient, then a crossmatch between the donor unit and the patient's blood type to confirm ABO compatibility is performed. Classically, this is performed as an immediate spin crossmatch, in which the patient's plasma is mixed at room temperature with an aliquot of RBCs from the prospective ABO-compatible unit and the absence of agglutination due to IgM isoantigens is verified. It is now acceptable practice, however, for blood banks to perform an electronic crossmatch, in which the laboratory information system runs through an algorithm to ensure that both patient and prospective RBC unit are compatible with regard to ABO and RhD.

The other type of crossmatch procedure is known as a full or Coombs crossmatch. This type of crossmatch is required when the patient has a historical or currently positive antibody screen, with or without an alloantibody of known specificity. Availability of antigen-negative units varies significantly depending on the specificity of the antibody (or antibodies) identified in the patient's plasma. After identifying prospective ABO/Rh-compatible units that are negative for the antigen(s) against which the patient has alloantibody(ies), a full crossmatch is performed. Patient plasma is incubated with RBCs from the selected units and testing performed from the immediate spin to the Coombs (IgG or antihuman globulin) phase to ensure compatibility beyond ABO. When the patient's antibody is reactive in the current sample, the Coombs crossmatch additionally ensures that the units lack the antigens for which the patient's serum contains preformed alloantibodies.

Incompatible crossmatches with multiple or all selected RBC units may be seen in a number of situations, most commonly in the presence of warm autoantibodies or panagglutinins. Understanding the reason for the incompatible crossmatch is critical to determining the risk vs the benefit of proceeding with transfusion of a crossmatch-incompatible RBC unit. Consultation with a blood bank physician is warranted in these situations.

- If a patient's plasma demonstrates the presence of clinically significant RBC alloantibodies, then ABO/Rh-compatible RBCs that lack the corresponding antigen(s) must be identified. These prospective units must then undergo a full or Coombs crossmatch with the patient's plasma.

Apheresis

Plasmapheresis

Common indications for therapeutic apheresis are given in Table 13-4. Plasma exchange typically involves centrifugation (less commonly filtration) of whole blood removed from the patient, selective removal of plasma which is replaced with defined volumes of replacement fluid (5% albumin, plasma, saline, or various combinations of these fluids), and return of cellular blood elements from the extracorporeal circuit to the patient. For a standard procedure, 1 plasma volume of patient plasma is removed and replaced, typically with 5% albumin. Plasma is used when factor replacement is needed, as in the case of TTP or perioperative plasma exchange. Centrifugal apheresis typically is performed in a continuous-flow fashion so that the patient remains euvolemic throughout the procedure.

Frequency and duration of therapy depend on the indication. Besides clinical trial data, the principles of apheresis that determine a treatment course include the theoretical efficiency of immunoglobulin removal. Approximately half of total IgG is intravascular. Because subsequent procedures remove plasma that has already had immunoglobulin removed, their efficiency is theoretically less than the first. Allowing time in between procedures allows for redistribution of IgG back into circulation, which increases the available IgG for removal. IgM is approximately 80% intravascular and is more efficiently removed than IgG. The adverse effects of plasma exchange are primarily driven by complications related to the central venous catheter, if needed; the risk of reactions with plasma replacement; vagal reactions; and reactions to the citrate or heparin used for anticoagulation.

Extracorporeal photochemotherapy (ECP or photopheresis) involves collecting peripheral blood mononuclear cells by apheresis (processing about one-third of the blood volume), adding a photoactivating agent (8-methoxysoralen) into the mononuclear cell suspension, treating the mononuclear cells with ultraviolet A light, and returning the treated cells to the patient. The process takes about 2 to 4 hours. ECP is an adjunctive therapy for erythrodermic cutaneous T-cell lymphoma. Patients typically are treated on 2 consecutive days every 4 weeks; the median time to response is 4 to 6 months. Response correlates with

KEY POINTS

- For blood products to be issued to a patient, the patient's ABO/Rh blood type must be determined and the patient's plasma must be screened for the presence of RBC alloantibodies that may have formed following a previous transfusion, HSCT, or pregnancy.
- If a patient's plasma lacks clinically significant RBC alloantibodies and he/she has no historical alloantibodies, then an immediate spin or electronic crossmatch is performed with prospective RBC units to ensure ABO blood group compatibility.

Table 13-4 Abbreviated list of therapeutic apheresis procedures grouped by American Society for Apheresis indication category

Disease/disorder	Procedure
Category 1. Accepted as first-line therapy, stand-alone or adjunctive	
Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)	Plasmapheresis
Cutaneous T-cell lymphoma; mycosis fungoides (erythrodermic)	Extracorporeal photopheresis
Familial hypercholesterolemia (homozygotes)	Selective absorption
Goodpasture syndrome	Plasmapheresis
Guillain-Barré syndrome	Plasmapheresis
Hyperviscosity in monoclonal gammopathies	Plasmapheresis
Myasthenia gravis	Plasmapheresis
Polycythemia vera	RBC exchange
Sickle cell disease (acute stroke treatment and prophylaxis)	RBC exchange
TTP	Plasmapheresis
ANCA-associated rapidly progressive glomerulonephritis	Plasmapheresis
Babesiosis, severe	RCD exchange
Antibody-mediated renal transplant rejection	Plasmapheresis
Category 2. Accepted as second-line therapy, stand-alone or adjunctive	
ABO-incompatible hemopoietic progenitor cell transplantation	Plasmapheresis
Catastrophic antiphospholipid syndrome	Plasmapheresis
Cold agglutinin disease, life-threatening	Plasmapheresis
Cryoglobulinemia	Plasmapheresis
Graft-versus-host disease (skin)	Extracorporeal photopheresis
Hyperleukocytosis/leukostasis	Leukapheresis
Myeloma cast nephropathy	Plasmapheresis
Pure RBC aplasia	Plasmapheresis
SCD (acute chest syndrome)	RBC exchange
Category 3. Role of apheresis is not established; decision making should be individualized	
Coagulation factor inhibitors	Plasmapheresis
Graft-versus-host disease (nonskin)	Extracorporeal photopheresis
Hyperleukocytosis/leukostasis (prophylaxis)	Leukapheresis
ITP (refractory)	Plasmapheresis
Malaria	Red blood cell exchange
Posttransfusion purpura	Plasmapheresis
Warm autoimmune hemolytic anemia	Plasmapheresis
Category 4. Evidence indicates apheresis to be ineffective or harmful	
Amyloidosis	Plasmapheresis
Rheumatoid arthritis	Plasmapheresis
SLE nephritis	Plasmapheresis

ANCA, antineutrophil cytoplasmic antibody; HUS, hemolytic-uremic syndrome; SLE, systemic lupus erythematosus.

the presence of circulating clonal tumor cells and a CD8-mediated antitumor response. ECP is also used to treat acute and chronic GVHD after allogeneic stem cell transplantation. The best response rates to ECP (~70%) are seen with chronic cutaneous GVHD in steroid-refractory cases.

LDL apheresis selectively removes LDL from plasma and is used to treat homozygous familial hypercholesterolemia or the heterozygous carrier refractory to maximal lipid-lowering drug therapy. The most common method used in the United States employs a dextran sulfate column to bind LDL and very-low-density lipoprotein, while sparing immunoglobulins and high-density lipoprotein (HDL). A typical procedure reduces LDL by 50% to 75% and is performed every 2 weeks. Some experts recommend that homozygous children start LDL apheresis around age 7 years to prevent premature atherosclerosis.

RBC exchange transfusion

RBC exchange transfusion therapy is performed most often in patients with SCD to prevent and treat acute complications of the disease, such as stroke and acute chest syndrome. Preoperative exchange transfusion is indicated when the preoperative hemoglobin is too high (typically >8.5 to 9 g/dL) to permit simple transfusion, or there is not sufficient time for serial transfusion to achieve a target sickle hemoglobin percent. Patients should be transfused with blood that is known to be negative for hemoglobin S. During RBC exchange utilizing an apheresis machine (erythrocytapheresis), the patient's erythrocytes are removed and replaced with donor erythrocytes while the patient's own plasma is continually returned to minimize disturbance of hemodynamic and coagulation parameters, although some platelets are removed during erythrocytapheresis. Another option is manual RBC exchange, in which manual phlebotomy is followed by infusion of donor RBCs. This is performed particularly in small children who cannot tolerate the volume shifts associated with apheresis and/or those who do not have the required vascular access for chronic automated RBC exchange. When manual exchange is done, careful attention must be paid to the potential for volume depletion due to excessive phlebotomy, and hypervolemia/hyperviscosity due to excessive RBC transfusion. The goal of exchange transfusion in most situations, such as acute chest syndrome, stroke, or preoperative exchange, whether performed manually or via automated RBC apheresis, is to achieve a hematocrit of 30% with a hemoglobin S of ≤30%.

PBSC harvesting

Mobilization refers to the technique of increasing the number of circulating progenitor cells in the peripheral blood. Chemotherapy and hematopoietic growth factors have

been the most commonly used mobilization agents, and newer agents can effectively augment mobilization.

It was noted in the 1970s that progenitor cells in peripheral blood increased up to 20-fold after chemotherapy for ovarian cancer. The introduction of hematopoietic growth factors in the late 1980s shortened the period of neutropenia after chemotherapy and was noted to increase circulating hematopoietic progenitors up to 1,000-fold. G-CSF downregulates the expression of adhesion molecules on the surface of HPSCs, progenitor cells, precursor cells, and mature neutrophils; and mobilizes clinically significant numbers of HPSCs into the peripheral blood.

Many mobilization regimens combine chemotherapy with growth factors. Cyclophosphamide followed by G-CSF is a commonly used protocol. The white cell count reaches 1×10^9 – 10×10^9 /L around day 11 to 13 after chemotherapy. Leukapheresis usually is scheduled for day 10 to 12 after chemotherapy. A mobilization regimen that has a predictable rebound phase allows for more efficient use of apheresis and stem cell-processing staff. The use of growth factor alone for mobilization avoids the risk of neutropenia with chemotherapy and is used in allogeneic donors. G-CSF at 10 µg/kg/day is a common mobilization regimen for allogeneic PBSC donors. With this regimen, leukapheresis begins on day 5, when the white cell count is 20×10^9 to 50×10^9 /L. The correlation is excellent between the number of CD34⁺ cells in the peripheral blood on the day of leukapheresis (or the preceding day) and the number of CD34⁺ cells that can be collected by apheresis. In general, for each 10^6 /kg target collection of CD34⁺ cells, the CD34⁺ cell count in the peripheral blood is 10×10^6 /L.

Although the administration of mobilizing doses of G-CSF can induce seemingly worrisome degrees of leukocytosis—transient peripheral blood leukocyte counts of 80,000/ μ L or higher are not uncommon—follow-up studies reported to date suggest that administration of short courses of G-CSF to healthy donors is not associated with any adverse long-term consequences. A rare complication of G-CSF mobilization is splenic rupture, which has been reported in healthy adult PBSC donors, most commonly after 5 daily doses of G-CSF.

Large-volume leukapheresis (LVL) refers to the processing of large volumes of blood (15 to 30 L over ~5 hours). LVL is indicated particularly in autologous transplant patients who do not mobilize CD34⁺ cells well. Data suggest that committed progenitor cells are recruited into the circulation during LVL. Although the magnitude of recruitment from LVL is small relative to the effects of chemotherapy and growth-factor mobilization, the 2 techniques can be combined for maximal benefit. LVL requires good venous access that would permit sufficient

flow rates. This may necessitate a central venous catheter. To minimize the risks of citrate toxicity, heparin may be added to the citrate; calcium supplementation is an alternative to heparin use. Platelet depletion is another predictable consequence of LVL.

A relatively common problem with PBSC harvesting is inadequate collection. The incidence of inadequate collection is much higher in heavily pretreated patients than in healthy donors. In healthy PBSC donors, increasing age, white ethnicity, and female sex were associated with lower post-G-CSF peripheral blood CD34⁺ counts, which correlate with lower CD34⁺ yields from collection. Risk factors for an inadequate autologous collection include multiple prior chemotherapeutic regimens, extensive prior radiation therapy, or administration of certain chemotherapeutic agents, such as fludarabine, lenalidomide, melphalan, chlorambucil, and nitrosoureas.

Plerixafor is a small-molecule reversible inhibitor of the chemokine receptor CXCR4 on stem cells; this inhibition facilitates HPSC egress from the bone marrow and is synergistic with the mobilizing effects of G-CSF. One dose of plerixafor given with G-CSF has been shown to successfully mobilize CD34⁺ cells in patients with multiple myeloma, Hodgkin disease, and non-Hodgkin lymphoma who failed previous mobilization attempts; plerixafor is uncommonly needed in healthy PBSC donors. Mobilization is maximal approximately 4 to 18 hours after dosing. The adverse effect profile of plerixafor (mostly gastrointestinal) does not appear to overlap with that of G-CSF. The use of plerixafor varies by center, with some centers routinely using it for mobilization to maximize yield and minimize apheresis collections.

KEY POINTS

- Apheresis selectively removes plasma, erythrocytes, or leukocytes for therapeutic benefit in a variety of hematologic diseases (eg, TTP, SCD, an HPSC collection).
- A variety of nonhematologic, antibody-mediated disorders can be successfully treated with apheresis to remove the causative antibodies, including Goodpasture syndrome, Guillain-Barré syndrome, and humoral rejection in organ transplantation.

Transfusion support in special clinical settings and pediatric populations

Patients who are candidates for HSCT

As autologous and nonmyeloablative HSCTs are being offered to a wider population of patients with hematologic

malignancies, clinicians must take into account the possibility that many patients newly diagnosed with hematopoietic malignancies may become potential candidates for HSCT during their clinical course. Since family members may be potential HSC donors, directed-donor transfusion products from relatives should be avoided to minimize the risk of HSCT graft rejection via alloimmunization to minor histocompatibility antigens. For newly diagnosed patients with acute leukemia who are likely to require HSCT, it may be useful to perform HLA typing early in the course of induction therapy. Thrombocytopenia during the postconditioning and pre-engraftment phase of HSCT is expected, and in most cases is easily managed by platelet transfusion support. Multiparity places females at risk of HLA alloimmunization, which can pose challenges in adequate platelet support during HSCT.

Hematopoietic stem cell infusion

In the setting of allogeneic HSCT, PBSCs and bone marrow typically are infused “fresh,” without cryopreservation. Cord blood and autologous PBSCs or bone marrow are nearly always cryopreserved before use because most transplantation preparative regimens require at least several days to administer before the stem cells can be infused. Optimal viability of the stem cells is achieved by controlled-rate freezing, using dimethyl sulfoxide (DMSO) as the cryopreservative. PBSCs typically are stored in the vapor phase of liquid nitrogen. Frozen aliquots of 50 to 75 mL are thawed sequentially during the infusion, at the bedside, or in the laboratory. This approach allows the maintenance of a relatively slow infusion rate while simultaneously maximizing PBSC viability by minimizing the interval between thawing and infusion of each aliquot. DMSO toxicity commonly manifests as flushing, nausea, vomiting, and blood pressure fluctuations; to minimize toxicity, the volume of DMSO infused should be limited to no more than 1 mL/kg at 1 sitting (which translates to 10 mL/kg of PBSCs for components that were cryopreserved with 10% DMSO).

CLINICAL CASE

A 56-year-old woman is being evaluated for matched HSCT from her brother, for high-risk acute myeloid leukemia. She is A positive, and he is O negative. She is enrolled in a nonmyeloablative conditioning protocol. On day 0, the PBSC is plasma depleted and infused without incident. On day 8, she is noted to have a hemoglobin of 6 g/dL (down from 9 g/dL the day before). She is asymptomatic and without any evidence of bleeding.

Transfusion support after HSCT

The intensity of transfusion support varies among conditioning regimens. In general, the transfusion needs are less in autologous transplantation and nonmyeloablative allogeneic conditioning regimens compared with allogeneic myeloablative regimens. In a hemodynamically stable patient without underlying cardiovascular disease, it is common practice to transfuse RBCs for a hemoglobin of 7 to 8 g/dL, although there has been no adequately powered RCT conducted to determine the optimal RBC transfusion threshold in this patient population. The landmark studies that support a platelet transfusion threshold of $10 \times 10^9/L$ were conducted in patients undergoing leukemia induction. Risk factors for platelet refractoriness such as fever, infection, bleeding, amphotericin, and vancomycin are common occurrences in HSCT patients. Veno-occlusive disease increases platelet consumption from endothelial damage and activation of VWF; portal hypertension and hypersplenism further increase platelet transfusion requirements.

Despite new antifungal agents, fungal infections in patients who have prolonged neutropenia remain problematic. There are case series of patients who received granulocyte transfusions as adjunctive therapy for refractory fungal (and bacterial) infections after HSCT and as secondary prophylaxis during HSCT after a prior episode of fungal infection. The use of granulocyte transfusions as primary prophylaxis after allogeneic HSCT produced a modest decrease in febrile days and antibiotic usage but no difference in treatment-related mortality in one study. Patients known to be HLA alloimmunized are at risk of greater pulmonary toxicity from granulocyte transfusions, although routine screening for HLA antibodies before granulocyte transfusions is not universal. Based on available data to date, routine use of granulocytes is not warranted given the minimal advantages to the recipients and potential risks of subjecting healthy donors to G-CSF and corticosteroids.

RBCs, platelets, and granulocytes must be irradiated before transfusion in HSCT recipients to prevent TA-GVHD. Some institutions with high-volume oncology and HSCT patient populations have elected to irradiate all platelet and RBC products to avoid the significant consequences of omitting this step. Irradiation shortens the shelf life of RBCs (but not platelets), necessitating attention to inventory management. Most centers recommend that HSCT survivors receive irradiated blood components indefinitely, in the absence of data that show the safety of nonirradiated components in long-term HSCT survivors.

Risk reduction for CMV infection is an important part of transfusion management in CMV-seronegative HSCT recipients. Leukoreduction filters achieve a 3- to 4-log reduction of leukocytes in blood products. A randomized comparison of leukoreduced vs CMV-seronegative blood components in CMV-seronegative HSCT recipients (with seronegative donors) found no significant difference in the incidence of CMV infection or disease as a composite outcome. In practice, many transplantation centers use prestorage leukoreduced blood components for CMV risk reduction.

ABO-incompatible HSCTs

Allogeneic HSCTs do not require ABO matching because ABO antigens are not expressed on pluripotent stem cells. Since the HLA and ABO genes are not coinherited, 2 siblings can have an identical HLA type but different ABO types. A report compiled from multicenter data reported to the International Blood and Marrow Transplant Research group included 3,000 patients with early-stage leukemia who underwent transplantation between 1990 and 1998 with bone marrow from an HLA-identical sibling donor. There was no difference in overall survival, transplantation-related mortality, and grade 2 to 4 acute GVHD in the ABO-identical vs ABO-mismatched groups. However, a single-institution study that focused exclusively on nonmyeloablative regimens found that ABO incompatibility was associated with increased nonrelapse mortality within the first year after HSCT. Similarly, the Japanese Marrow Donor Program has reported increased acute GVHD in ABO-mismatched unrelated donor transplants and increased transplantation-related mortality in the subset that received nonmyeloablative conditioning. In the unrelated donor setting, there may be multiple potential HLA matches for any given patient, and in light of these findings, ABO compatibility is a secondary consideration along with donor sex, age, and CMV status, that contribute to donor choice. In all cases of ABO-incompatible HSCT, the blood bank must be aware of the clinical situation and receive serial samples in order to correctly report ABO type and determine when the ABO switch has occurred.

In major ABO-incompatible HSCT, the recipient has preformed antibodies against donor red cell A or B antigens. Major ABO-incompatible HSCT can lead to acute hemolysis during or immediately after graft infusion. RBC depletion can be performed prior to infusion but may also reduce the stem/progenitor dose of the graft due to processing loss. Apheresis collections typically do not require RBC depletion. Major ABO incompatibility can also lead to delayed RBC recovery but has not been shown to im-

pact overall engraftment. Delayed RBC engraftment and prolonged anemia occur more frequently in major ABO-incompatible HSCT when performed with reduced-intensity conditioning regimens due to the persistence of recipient-derived plasma cells. The incidence is approximately 10%, and there is an inverse correlation between ABO isoantibodies and reticulocyte counts.

Minor ABO-incompatible HSCT occurs when the donor anti-A or anti-B antibodies are directed against the recipient's RBC antigens. The risk of graft infusion-associated hemolysis is low but can be prevented by plasma volume reduction of the donor product. Passenger lymphocyte syndrome is a complication in which transplanted donor lymphocytes produce new antibodies 1 to 3 weeks following HSCT, which can result in persistent hemolysis until the recipient RBCs are no longer produced. Passenger lymphocyte syndrome occurs more commonly with T-cell-depleted marrows, PBSC vs a marrow source, the use of cyclosporine alone (without methotrexate) for GVHD prophylaxis, and reduced-intensity conditioning regimens. Some centers perform periodic DAT screening in minor ABO-mismatched HSCT recipients, although the utility is not clear. It may be prudent to maintain a higher transfusion threshold in minor-mismatch recipients during the at-risk period after transplantation. Massive hemolysis may be treated by erythrocyte exchange transfusion using RBCs compatible with both donor and recipient types.

HSCT recipients with non-ABO alloantibodies have undergone transplantation with antigen-positive grafts using the same principle of RBC depletion of the product. An Rh(D)-positive recipient who undergoes HSCT from an Rh-negative donor may develop anti-D as the donor lymphocytes respond to the residual Rh-positive RBCs. Patients with SCD undergoing HSCT may present a challenge if they have developed multiple RBC alloantibodies or an antibody to a high-incidence antigen. The optimal time to discontinue antigen-negative blood is unknown, but one strategy is to wait until chimerism tests show 100% donor lymphocytes because residual recipient lymphocytes may resume production of RBC alloantibodies with donor specificity. Alloimmune hemolysis should be considered in the posttransplantation patient with hyperbilirubinemia. Finally, HSCT patients whose disease relapses may revert to the recipient ABO/Rh type. The transfusion service must be alert to subtle changes in mixed-field agglutination in ABO blood grouping during these situations. Table 13-5 provides useful guidelines for the selection of the appropriate blood group type for RBCs, platelets, and plasma for donor-recipient ABO-HSCT incompatibility.

Table 13-5 Peritransplant guidelines for blood component selection in ABO-incompatible HSCT

Recipient blood type	Donor blood type	RBC transfusion	Platelet/plasma transfusion
O	A	O	A or AB
O	B	O	B or AB
O	AB	O	AB
A	B	O	AB
A	AB	A or O	AB
A	O	O	A or AB
B	A	O	AB
B	AB	B or O	AB
B	O	O	B or AB
AB	A	A or O	AB
AB	B	B or O	AB
AB	O	O	AB
Rh neg	Rh pos	Rh neg	Rh pos or Rh neg
Rh pos	Rh neg	Rh neg	Rh pos or Rh neg

neg, negative; pos, positive.

KEY POINTS

- For optimal cell viability, frozen aliquots of HPSCs must be thawed rapidly and infused into the patient without delay, sometimes leading to DMSO toxicity.
- RBC, platelet, and granulocyte products administered to HSCT recipients must be irradiated to minimize the risk of potentially fatal TA-GVHD and leukoreduced to minimize the risks of CMV transmission and alloimmunization to HLA antigens.
- Donor-recipient mismatches involving the ABO system usually are well tolerated but occasionally can cause delayed alloimmune hemolytic anemia or pure RBC aplasia.

Pediatric transfusion issues

Hemolytic disease of the fetus and newborn

HDFN (or erythroblastosis fetalis) is most commonly due to maternal-fetal mismatches involving the Rh or ABO antigens. For non-ABO antigens, exposure to antigen-positive fetal RBCs can cause an antigen-negative mother to mount an antibody response. Maternal IgG antibodies can cross the placenta and cause passively acquired immune-mediated hemolytic anemia in the fetus, potentially leading to profound anemia and in severe cases, hydrops fetalis and fetal demise. Maternal antibodies formed in response to sensitization by RBC transfusion can also lead to HDFN. The incidence of severe HDFN has been

reduced dramatically with the use of antenatal and peripartum administration of RhIg to Rh(D)-negative mothers, which abrogates the maternal immune response to primary exposure to D antigen. Lack of access to prenatal care is now a leading cause of HDFN due to anti-D. Nevertheless, most cases of HDFN are now attributed to sensitization to Rh antigens other than D, as well as K (Kell blood group system) and ABO. Although severe examples have occurred rarely, ABO HDFN is typically characterized by hyperbilirubinemia with mild anemia (if any); the mother is usually group O with IgG anti-A,B alloantibodies (an antibody with cross-reactivity to both A and B antigens), and the infant is most commonly group A.

Intrauterine transfusion

Due to widespread use of RhIg for prophylaxis against D sensitization from pregnancy, the need for intrauterine transfusion (IUT) due to HDFN is uncommon, and technical expertise is currently concentrated in centers that specialize in high-risk obstetrics. In a sensitized pregnancy, middle cerebral artery Doppler ultrasound and amniotic fluid studies guide the need for fetal blood sampling, which may be performed after 20 weeks of gestation. Blood is prepared for IUT if the fetal hematocrit is <25% to 30%. Group O, D-negative RBCs lacking the implicated RBC antigen are selected; some centers match the extended maternal RBC phenotype beyond the implicated antigen. Maternal serum or plasma is used for crossmatching. A fresh RBC unit (less than 5 days old) is typically used, either citrate-phosphate-dextrose-adenine (CPD-A) unit (without additive solution) or an additive solution unit with the supernatant removed. The RBCs should be irradiated to prevent TA-GVHD since the fetal immune system is immature; leukoreduced or from a CMV-seronegative donor to provide a CMV-safe component, and negative for sickle hemoglobin. The selected RBC unit is usually washed and concentrated to the volume and hematocrit specified by the obstetrician performing the procedure. Once IUT is initiated, it is repeated every 3 to 4 weeks until 35 weeks of gestation to maintain a minimum fetal hematocrit at approximately 25%. Neonates who have undergone IUT type as O negative; such neonates may have suppressed erythropoiesis and/or persistent maternal antibody, which necessitate postnatal transfusion support for up to 3 months. Complications of IUT are related primarily to the technical complexity of vascular access.

Neonatal exchange transfusion

Advances in phototherapy and antenatal care have made exchange transfusion for HDFN, or hyperbilirubinemia due to other causes, an uncommon occurrence. Appropriate

unit selection follows the same principles for IUT described previously (ie, fresh O-negative unit, negative for any offending antigen, crossmatched against maternal serum, leukoreduced, irradiated, and hemoglobin S negative). In addition, the RBCs are concentrated and reconstituted with group AB plasma, typically in a 1:1 ratio to produce a unit of reconstituted “whole blood” (hematocrit 50%) for the exchange. A double-volume exchange removes approximately 85% of the neonate’s antigen-positive RBCs but is less efficient in lowering plasma bilirubin. Complications of exchange transfusion include hypocalcemia, dilutional thrombocytopenia, and catheter-related complications such as thrombosis, infection, or bleeding.

Alloimmune cytopenias in the fetus or newborn

Analogous to HDFN, maternal-fetal mismatches involving platelet-specific or neutrophil-specific antigen systems may result in NAIT or NAIN, respectively. The target antigens are quite diverse but are often membrane glycoproteins. The most common antibody specificity in NAIT in European backgrounds targets HPA-1a (PL^{A1}), which resides on the platelet fibrinogen receptor GPIIb/IIIa, although numerous other platelet antibody specificities have been reported. It is worth noting that while HDFN due to maternal sensitization through pregnancy typically occurs with the second pregnancy, NAIT may occur during a first pregnancy. NAIN often is due to fetal-maternal mismatches involving the neutrophil-specific NA-1/NA-2 system.

Prenatal management of these disorders often includes maternal IVIg to decrease placental transfer of antibodies and reduce cellular destruction in the fetus. Infants with NAIT are at risk of life-threatening bleeding such as intraventricular hemorrhage, which can occur in utero; and therefore, infants should be screened with a head ultrasound immediately after birth. Transfusion support of NAIT is initiated with random donor platelets, which usually results in an adequate platelet increment in the majority of cases. If subsequent platelet transfusion is needed, washed irradiated maternal platelets that are negative for the target antigen can be obtained but in the majority of cases, infants with NAIT respond to random donor platelets. Maternal platelets are negative for the target antigen in question and may abrogate the wait for the time-consuming identification of platelet alloantibody specificity. Some blood centers have registries of specific platelet antigen-negative donors available for platelet donation if required. IVIg is a therapeutic option if the bleeding is mild to moderate.

Maternal ITP or autoimmune neutropenia (AIN) can cause passively acquired immune-mediated thrombocytopenia or neutropenia, respectively, in the fetus. It is important to screen for these disorders in the mother. The currently available assays for antiplatelet antibodies and antineutrophil

antibodies are not highly sensitive or specific and the diagnosis or exclusion of ITP or AIN should not be based solely on the results of antibody assays.

RBC transfusion in preterm neonates

The physiologic anemia of infancy occurs at 8 to 12 weeks, and the nadir hemoglobin is rarely <9 g/dL. Among preterm infants, this decline occurs at an earlier age, and the nadir is 7 to 8 g/dL, which may also be compounded by iatrogenic phlebotomy. The blood loss through cumulative phlebotomy in a preterm infant's first weeks of life can be significant. Judicious laboratory monitoring can help minimize transfusion requirements. Delaying umbilical cord clamping for 30 to 60 seconds for infants who do not require immediate resuscitation has been advocated by some to be the first step in counteracting the anemia of prematurity. Erythropoietin has limited efficacy in preterm infants and has been associated with an increased risk of retinopathy of prematurity. Limited donor exposure can be achieved by dedicating a fresh O-negative RBC unit (≤ 7 days old) to 1 or 2 preterm infants and used exclusively to transfuse those infants.

Two randomized clinical trials of restrictive vs liberal transfusion criteria used transfusion thresholds that varied with patients' postnatal age, and respiratory and medical status. A stable older infant in the restrictive arm, for instance, would be transfused at a hemoglobin level of approximately 7.5 g/dL; a younger mechanically ventilated preterm infant would be transfused at a hemoglobin level of approximately 11.5 g/dL. In both trials, the number of donor exposures from RBC transfusions alone was not reduced by restrictive transfusion criteria, presumably reflecting the efficacy of using dedicated donor units; only 1 of the 2 trials demonstrated that a restrictive transfusion threshold increased the percentage of infants who avoided transfusion altogether (from 5% to 11%).

Most U.S. centers routinely irradiate all cellular components for neonates for a variable period of time after birth (typically 4 to 6 months). Other centers use criteria based on gestational age and birth weight. In addition, leuko-reduced cellular components are used to reduce the risk of CMV transmission. Some centers may use CMV-seronegative components for specific subgroups, such as neonates weighing $<1,200$ g. The quantity of additives in stored RBCs, such as citrate, adenine, and mannitol, is far less than levels believed to be toxic. Washing to reduce the potassium load is not indicated in small-volume transfusions; however, use of fresh or washed RBCs may be performed for large-volume transfusion. RBCs for neonates requiring large-volume transfusion should be irradiated as close as possible to the time that they are transfused to avoid significant increases in extracellular potassium levels. Although

2,3-DPG is depleted in stored RBCs, it is rapidly regenerated after transfusion; infants given stored RBCs have stable 2,3-DPG levels after small-volume transfusions. In the ARIPI double-blind RCT, use of fresh RBCs (mean age 5.1 days) compared with standard-issue RBCs (mean age 14.6 days) did not improve outcomes in premature, very-low-birth-weight infants requiring transfusion.

Other component therapy in neonates

Newborns may require plasma transfusion, most commonly for DIC secondary to sepsis; 10 to 15 mL/kg produces a 15% to 20% increase in factor levels, assuming ideal recovery. If cryoprecipitate is required for persistent hypofibrinogenemia despite plasma transfusion, a dose of 1 unit should produce a 100 mg/dL increase in fibrinogen (in older infants, the cryoprecipitate dose is 1 unit per 5 to 10 kg of body weight).

Neonatal thrombocytopenia is common in preterm neonates, occurring in 22% of infants in one series. It is frequently a sign of sepsis, severe inflammation, and perinatal asphyxia/placental insufficiency. Prophylactic transfusions often are recommended in neonates with platelet counts $<20,000$ to 30,000/mL if otherwise stable. In unstable neonates or those requiring invasive procedures, platelets are often transfused to maintain a count of $\geq 50,000/\mu\text{L}$. The usual platelet dose in neonates is 10 to 15 mL/kg or 1 equivalent unit per 5 to 10 kg. Similar to RBCs, infants requiring significant platelet transfusions may also receive aliquots from a dedicated apheresis platelet unit to reduce donor exposures, although the shelf-life of platelets is quite short. Platelets should be ABO identical to avoid the transfusion of minor incompatible plasma into the small blood volume of a neonate. If ABO-identical (or group AB) platelets are not available, platelets can be washed, or volume reduced to remove incompatible plasma. Routine washing or volume reduction of platelets is not necessary or recommended because the procedure can jeopardize platelet quality.

KEY POINTS

- The immune system in the fetus and in neonates up to the age of 4 months is immature and typically not capable of generating antibody responses to transfusions. Thus, the most crucial compatibility issues involve the passive transfer of antibodies from the mother to the fetus, as well as maintaining ABO compatibility between the donor and the fetus or neonate.
- Current blood banking practice attempts to limit the number of donor exposures to fetal and neonatal patients by using multiple transfusion aliquots from single blood products.

Pediatric transfusion beyond the neonatal period

The posttransfusion long-term survival rate in pediatric transfusion recipients is much higher than in adults, so the principle of minimizing donor exposure, which carries risks of transfusion-transmitted disease (involving known and unknown infectious agents), continues beyond the neonatal period. A multicenter trial of restrictive vs liberal transfusion thresholds (7 g/dL vs 9.5 g/dL) in pediatric ICUs found that a restrictive transfusion strategy was non-inferior in the primary outcomes (28-day mortality and new or progressive multiorgan dysfunction) and successfully avoided transfusion in 54% of patients (compared with 2% in the liberal transfusion group). The older child or adolescent undergoing elective surgery may benefit from judicious use of autologous blood donation and intraoperative cell salvage in an integrated blood-conservation approach.

Autoimmune hemolytic anemia

CLINICAL CASE



A 69-year-old woman presents with an Hb of 6 g/dL. The DAT is positive for IgG and negative for complement, indicating that circulating RBCs are coated with IgG. Her reticulocyte count is <1%. She has never been transfused and has never been pregnant. The patient is started on prednisone for treatment of presumed warm (IgG-mediated) AIHA. Because of shortness of breath, an RBC transfusion is ordered. Multiple RBC crossmatches are incompatible. Two units of crossmatch-incompatible leukoreduced RBCs are transfused. The peripheral blood hemoglobin concentration increases to 8 g/dL, and she experiences no untoward reactions.

Transfusion in patients with AIHA can be challenging. Autoantibodies to RBCs can result in multiple incompatible crossmatches, which may lead blood banks to inform clinicians that no compatible RBC units are available. FDA regulations require the patient's physician to provide written consent to release incompatible units, which makes many clinicians uncomfortable. If the patient has not been previously transfused or pregnant, alloantibodies to non-ABO antigens are unlikely to be present, and patients can usually be transfused safely with ABO-compatible blood. Even in patients who have been previously transfused or pregnant, withholding transfusions due to incompatible crossmatches may preclude the administration of lifesaving transfusions.

Multiply transfused patients with AIHA are at risk of alloimmunization. Thus, if a patient has received a transfusion or been pregnant, the transfusion service must per-

form specific testing to determine whether alloantibodies are present concurrently with the panagglutinating autoantibodies associated with AIHA. The term *panagglutinating* refers to the fact that most autoantibodies that cause AIHA agglutinate most or all RBCs, including reagent RBCs and RBCs for transfusion because the antigenic target is typically an antigen present on the RBCs of a large proportion of the population. This antigen is often a common Rh epitope.

Some transfusion services routinely perform extended RBC typing for patients with AIHA at the time of diagnosis. Extended RBC typing can facilitate new alloantibody identification following transfusion. DNA-based methods are preferable, when available, due to the interference of a positive DAT with serologic typing, and the additional antigen information provided by these methods. In rare situations, when the presence of underlying alloantibodies cannot be excluded, transfusion of RBC units phenotypically similar to the patient's own extended RBC phenotype (Rh, K, Jk, Fy, Ss antigens) may help reduce the risk of hemolysis due to alloantibodies in this setting.

The technique for detecting alloantibodies in the presence of autoantibodies is called adsorption. With the autoadsorption technique, an aliquot of the patient's plasma is adsorbed repeatedly with the patient's own RBCs. This step removes autoantibody on the autologous RBCs and leaves any RBC alloantibody in the plasma. The remaining plasma is then tested for alloreactivity with a panel of donor RBCs in a standard antibody screen. The technique is time-intensive, and results can take several days if the antibody specificity is unusual. If the patient has undergone transfusion recently, autoadsorption cannot be reliably interpreted because the transfused RBCs present in the patient's circulation could adsorb the very same alloantibodies that the laboratory is attempting to detect. In this situation, a method called differential alloadsorption is used. Differential alloadsorption, sometimes called triple adsorption, involves adsorbing aliquots of patient serum against RBCs of defined phenotypes to produce several adsorbed sera that give differential reactivity in standard antibody screens. The differential reactivity results from the fact that alloantibodies are left behind in the serum following the adsorption if the adsorbing cells are negative for the antigen in question. Since most warm-reacting autoantibodies react with RBC surface determinants that do not vary among individuals (ie, common antigens), adsorption with RBCs of different phenotypes removes the autoantibody but, depending on the phenotype, either removes or fails to remove alloantibody. For example, if the patient's serum contains an anti-Jk^a antibody, both the autoantibody and the anti-Jk^a antibody are adsorbed by Jk^a-positive adsorbing cells, but only the autoantibody is

adsorbed by Jk^a-negative adsorbing cells. The presence of the anti-Jk^a in the patient's serum then can be deduced by demonstrating that the aliquot of the serum that was adsorbed by Jk^a-positive cells is nonreactive in a standard antibody screen, whereas the aliquot of serum that was adsorbed by Jk^a-negative cells reacts only with Jk^a-positive cells in a standard antibody screen.

Warm-reacting autoantibodies occasionally demonstrate preferential reactivity against certain antigens. The apparent specificity demonstrated by autoantibodies is often directed to an antigen in the Rh blood group system, most commonly to the e (little e) antigen. For transfusion, the survival of antigen-positive donor RBCs usually does not differ from that of the patient's own RBCs; however, in some cases, RBCs that do not express the target antigen may survive longer following transfusion.

In patients with clinically significant cold-reacting autoantibodies, such as anti-I, RBCs lacking the offending antigen are often not available. Blood transfused through a blood warmer usually survives adequately if the patient is kept warm while other forms of treatment, such as cytotoxic chemotherapy or plasmapheresis, are instituted. If requested, a blood bank work-up of the cold-reacting autoantibodies can include the performance of a thermal-amplitude determination in which RBC binding in vitro to the patient's autoantibodies is assessed as a function of temperature (eg, at 4°C, 22°C, 30°C, and 37°C). Autoantibodies that are reactive at body temperature are considered clinically significant. The results of such tests can give the clinician a sense of the potential clinical significance of the autoantibodies *in vivo* at body temperature.

In the clinical case described above, the patient's reticulocyte count was low. A substantial minority of patients manifest at least transient reticulocytopenia early in the course of AIHA, a phenomenon that may be due to autoantibody titers that increase more quickly than the bone marrow's reticulocyte response or due to rapid destruction of reticulocytes by the autoantibody. Reticulocytopenia with brisk AIHA is an emergency situation and transfusion should not be delayed.

KEY POINTS

- RBC transfusions in patients with life-threatening AIHA should not be withheld simply because all available units are crossmatch incompatible. Consultation with a transfusion medicine physician may be helpful for assessment of transfusion risks in patients with complex serologic work-ups.
- Special blood bank techniques are available to minimize the risk of transfusion in patients with AIHA.

Autoimmune and consumptive thrombocytopenias

Transfusion of platelets in patients with ITPs is usually not indicated since the transfused platelets are also destroyed by the antibody. As is the case with AIHA, the autoantibody in ITP often reacts with public antigens. Platelet transfusion in patients with ITP is reserved for life-threatening hemorrhages and for major surgery. Administration of IVIg may improve the survival of transfused platelets in patients with ITP, and the administration of IVIg or continuous infusions of platelets has been used in patients with life-threatening hemorrhage and those undergoing major surgery. Elective splenectomy typically is managed with preoperative IVIg or a pulse of corticosteroids. Intravenous Rh(D) IgG can be administered more quickly than IVIg, but its use is limited to Rh(D)-positive, nonsplenectomized patients.

Except in life-threatening bleeding situations, such as intracranial hemorrhage, platelet transfusions should be avoided in other consumptive thrombocytopenias as well (such as TTP and heparin-induced thrombocytopenia), which may exacerbate the underlying thrombotic process.

Sickle cell disease

Indications for transfusion in SCD include stroke, acute chest syndrome, aplastic crisis, and preoperative preparation to reduce the risk of postoperative respiratory complications and vaso-occlusive events. Patients who require chronic transfusion therapy accumulate iron much less rapidly if the transfusion occurs in the form of exchange procedures rather than simple transfusions; although exchange transfusion carries the risk of additional donor exposures and requires adequate vascular access.

Patients with SCD have a higher risk of alloimmunization compared to other patient populations who also require frequent or chronic transfusion. Approximately ~25% to 50% become alloimmunized, depending on the level of antigen matching, cumulative exposure history, and other recipient factors. Patients with SCD account for more than half of the requests for rare phenotype blood received by the American Red Cross Rare Donor Registry, which collects and distributes blood from donors with rare or uncommon phenotypes. One major reason for the high rate of alloimmunization is the differences in RBC antigen phenotypes between patients who are primarily of African descent and donors who are primarily of European backgrounds (in the United States and United Kingdom). Patients with SCD may also have a higher intrinsic immune responsiveness to blood group antigens due to underlying inflammation. Consistent with this, patients

with SCD are more likely to form alloantibodies when transfused during hospitalizations for acute chest syndrome or painful vaso-occlusive episodes.

Alloimmunization can be associated with delayed hemolytic transfusion reactions with varying degrees of anemia, hyperbilirubinemia, and/or pain. Hyperhemolysis is a transfusion-related complication observed in patients with SCD, often presenting with severe anemia and reticulocytopenia 7 to 10 days after transfusion. The hematocrit is lower than the pretransfusion level, suggesting potential destruction of autologous RBCs. The lower hematocrit may also reflect a lower erythropoietic drive following transfusion. The DAT is often negative, and new alloantibodies may or may not be detectable. It is important to recognize this syndrome because its management consists of the judicious avoidance of additional transfusions in the face of severe anemia, corticosteroids, IVIg, and erythropoietin. There is an association among DHTRs, the onset of sickle cell vaso-occlusive crises, and the occurrence of other SCD complications. A transfusion reaction should always be considered in the differential diagnosis of a patient with SCD with fever, worsened hyperbilirubinemia, or pain.

The development of alloantibodies in patients with SCD can also be associated with autoantibody formation, which further complicates transfusion therapy. The prevalence was 8% in one pediatric series of patients with SCD; about half the patients with autoantibodies had evidence of hemolysis, often associated with a positive DAT for complement.

Clinical practices for prevention and management of alloimmunization in patients with SCD are varied. Optimal management includes extended antigen typing for the most important antigen systems in addition to ABO and RhD, including Rh (C, c, E, e) Kell (K), Kidd (Jk^a, Jk^b), Duffy (Fy^a, Fy^b), and MNSs. DNA-based methods may be preferable, particularly in recently or multiply transfused patients. Extended RBC phenotyping facilitates identification of antibody specificities when a new antibody is detected. Prophylactic C, E, and K matching is recommended for all patients with SCD. Some institutions also match c and e antigens, while less commonly, extended RBC matching is performed and includes Jk^a/Jk^b, Fy^a/Fy^b, and Ss in addition to C, E, and K. It is not feasible to routinely match all antigens that the patient lacks with typical donor inventories. The adoption of high-throughput blood group genotyping platforms by more blood centers facilitates extended blood group matching between blood donors and patients with SCD, particularly when coupled with minority donation recruitment efforts.

KEY POINTS

- Patients with SCD should receive ABO, D, C, E, and K-matched RBCs to minimize alloimmunization.
- There is a high incidence of DHTRs in SCD and the potential for life-threatening hyperhemolytic transfusion reactions.
- Transfusion is the standard of care for the prevention and treatment of select complications of SCD (eg, stroke, acute chest syndrome, and aplastic crisis).

Massive transfusion

Massive transfusion is defined as the replacement of 1 blood volume within 24 hours, or transfusion of a certain number of RBC units within 4 to 6 hours, typically in the setting of severe trauma or major surgery. Coagulopathy of massive transfusion is multifactorial and includes hypothermia, acidosis, dilutional effect of blood loss, inadequate coagulation factor replacement, reduced hepatic synthesis of coagulation factors in massive hepatic injury, DIC from hypotension and tissue injury, and consumption of coagulation factors or platelets.

Both laboratory tests and transfusion volume may not correlate well with the severity of bleeding. Thrombocytopenia is the most frequent abnormality associated with massive transfusion. When transfusions of 1.5 to 2.0 blood volumes are administered over 4 to 8 hours, the mean reduction in the peripheral blood platelet count is approximately 50%. One strategy for management of massive transfusion is to allow the prothrombin time, activated partial thromboplastin time, plasma fibrinogen level, and platelet counts to guide component replacement therapy. In practice, many trauma centers have adopted an empiric preemptive approach to prevent coagulopathy based on military experience using early aggressive plasma transfusion and/or fixed ratios of blood components in standardized massive transfusion protocols. Patients undergoing massive transfusion need to be monitored for electrolyte disturbances such as hypocalcemia (citrate in the anticoagulant used for all blood components binds free calcium), hyperkalemia or hypokalemia, and metabolic alkalosis (from citrate metabolism).

Cardiopulmonary bypass

Alterations in the laboratory parameters of hemostasis are observed in virtually all patients undergoing open-heart surgery and extracorporeal circulation. Less than 10% of these patients experience severe bleeding, however, and during the history of cardiopulmonary bypass procedures,

blood usage for surgery involving extracorporeal circulation has decreased markedly. Dilution by priming the extracorporeal circuit with nonblood solutions may reduce the platelet count by as much as 50%. Platelet dysfunction results from platelet contact with the surfaces of extracorporeal circuits, including pumps and ventricular assist devices. Preoperative therapy with antiplatelet agents, such as aspirin, clopidogrel and GPIIb/IIIa inhibitors, exacerbates platelet dysfunction. Changes in platelet function due to exposure to the extracorporeal circuit may persist for several hours after discontinuation of bypass. Although plasma coagulation factor levels are diluted by nonblood priming solutions, coagulation factor levels ordinarily remain above the minimal level needed for hemostasis; that is, approximately 50% of the normal factor levels. The extracorporeal circuit is not thought to consume clotting factors directly. Consequently, platelet transfusion to correct quantitative or qualitative platelet defects is the mainstay of treatment of nonsurgical bleeding associated with cardiopulmonary bypass procedures. In addition, because platelet products contain significant quantities of plasma, platelet transfusion may be effective even when the primary laboratory abnormalities appear to be coagulation factor related, although particular attention should also be paid to fibrinogen replacement in this setting.

Routine transfusion of platelets to patients who are not bleeding and are not severely thrombocytopenic is not indicated. Thromboelastography offers whole blood-based coagulation testing that can localize the coagulation defect to a deficiency in platelets, coagulation factors or fibrinogen, or excessive fibrinolysis.

urine, and laboratory evidence of intravascular hemolysis. ABO isoantibodies are complement-fixing and lead to the intravascular destruction of the transfused RBCs, which can manifest as hemoglobinemia and hemoglobinuria. Often, fever is the only initial sign. Activation of complement leads to the release of cytokines, including tumor necrosis factor, accounting for fever and chills. The serologic hallmark of an acute hemolytic reaction is a positive DAT that demonstrates both IgG and complement on the surface of the recipient's circulating RBCs. DIC may occur.

Patient misidentification due to systems errors or failure to follow established hospital procedures remains the most common cause of acute hemolytic transfusion reactions; therefore, the importance of definitive bedside patient identification, both at the time that type and screen specimens are obtained and at the time that the product is ready to be administered, cannot be overemphasized. Barcode and radio-frequency chip technologies to ensure correct patient identification have been shown to reduce the risk of mistransfusion.

Acute hemolytic reactions can occur after platelet transfusions, typically involving a group A patient receiving group O platelets that contain high-titer anti-A antibody.

Treatment of acute intravascular hemolytic reactions is supportive and includes fluids and vasopressors for hypotension and maintenance of urine output. Even though febrile transfusion reactions are common, it is always important to stop transfusions at the first sign of fever, because fever may be the first sign of an incompatible transfusion.

Transfusion risks

CLINICAL CASE



Shortly after initiation of an RBC transfusion, a 63-year-old patient with melena develops pain at the infusion site followed by dyspnea, fever, chills, and low back pain. His urine is noted to be red and his plasma demonstrates free hemoglobin. Repeat testing of both the RBC product and the patient reveals that the product is type A, the patient is type O, and the crossmatch is incompatible.

Delayed hemolytic transfusion reactions

DHTRs occur when a patient develops an alloantibody to an RBC antigen following pregnancy, transfusion, or HSCT, but the titer of the antibody falls to below the detectable limit, resulting in an apparently negative antibody screen before a subsequent RBC transfusion. Following the subsequent transfusion, the patient develops an anamnestic immune response to the mismatched antigen, leading to delayed antibody-mediated destruction of the transfused RBCs. Clinical symptoms of hemolysis including fever, anemia, and jaundice develop 7 to 10 days after the transfusion; however, the link to the preceding transfusion is not always obvious. If blood bank testing is performed at this point, the DAT is often positive for IgG, with or without complement depending on the causative antibody. Because a positive DAT can be nonspecific, an eluate may be performed to remove the IgG antibody coating the circulating RBCs in order to identify the

Acute hemolytic transfusion reactions

The patient in this clinical case illustrates the typical presentation of an acute hemolytic transfusion reaction: pain at the administration site, fever, chills, back pain, dark

specificity. The antibody screen may also demonstrate the presence of a new antibody, although this may lag behind the positive DAT by a few days. Hemolysis is usually IgG mediated and thus extravascular, although IgG alloantibodies to Kidd blood group antigens may fix complement and cause intravascular hemolysis. Hemoglobinuria may occur, and occasional instances of severe complications such as acute renal failure or DIC have been reported. The antibodies most often implicated in DHTTR are directed against antigens in the Rh (34%), Kidd (30%), Duffy (14%), Kell (13%), and MNSs (4%) antigen systems.

Febrile nonhemolytic transfusion reactions

Multiparous women and multiply transfused patients develop antileukocyte antibodies that cause febrile nonhemolytic reactions to RBC or platelet transfusions. In addition, during the storage of blood, clinically significant quantities of cytokines (IL-1, IL-6, IL-8, and tumor necrosis factor) are sometimes liberated from donor-derived leukocytes present in platelet and RBC products. Prestorage leukoreduction, as opposed to poststorage bedside leukofiltration, reduces the accumulation of these biologic mediators and the incidence of febrile, hypotensive, or hypoxic transfusion reactions.

Febrile nonhemolytic transfusion reactions typically manifest during or within 4 hours of transfusion with fever (defined as an increase in temperature of 1°C above the patient's baseline, typically to >38°C) with or without chills and/or rigors. Such reactions may also manifest primarily with chills and/or rigors with a minimal or absent febrile component, particularly in patients receiving an antipyretic. Symptoms are usually self-limited and respond to symptomatic therapy, which includes antipyretics for fever and chills and meperidine for rigors. While transfusions are without undue risk in most cases, the main concern is that an elevation in temperature during a transfusion, although most likely the result of this innocuous febrile transfusion reaction or the patient's underlying medical condition, cannot be distinguished from an evolving life-threatening acute hemolytic or septic transfusion reaction in which fever can be the only clue. Completing a blood bank evaluation to rule out hemolysis is necessary with fevers occurring during transfusion. The increasing adoption of universal leukoreduction has been associated with a significant reduction in febrile nonhemolytic transfusion reactions, but no change in the incidence of allergic reactions. Studies do not demonstrate a benefit for the routine use of premedication to prevent febrile-nonhemolytic transfusion reactions, but many clinicians premedicate if a fever would change clinical management (eg, in the setting of neutropenia).

Allergic transfusion reactions

Minor allergic reactions manifested by urticaria and pruritus are frequent. Antihistamines generally alleviate symptoms of allergic reactions, but they have not been shown to prevent them. Many urticarial reactions do not recur with subsequent transfusions. If a recipient experiences multiple urticarial reactions, premedication with antihistamines (particularly non-sedating ones) can be considered. Washed products resuspended in albumin and/or saline may be considered in severe cases. Although removing plasma through washing mitigates allergic reactions, washing platelets impairs platelet function and leads to accelerated clearance after transfusion.

Severely IgA-deficient patients may make anti-IgA antibodies that can cause anaphylactic reactions, but this is a rare occurrence. Considering the high prevalence of IgA deficiency with anti-IgA antibodies (~1 in 1,200) and considering that passively transfused anti-IgA antibodies do not cause allergic reactions, the role of anti-IgA in the pathophysiology of recurrent and severe allergic transfusion reactions is not clear, and likely overestimated. Most fatal anaphylactic transfusion reactions are not due to IgA deficiency or anti-IgA. Washed RBCs, washed platelets, and/or platelet and plasma products from IgA-deficient donors should be transfused only when a patient has severe IgA deficiency (<0.05 mg/dL) and a concern for anaphylactic reactions. Most IgA-deficient patients, even those with anti-IgA, have no adverse reactions to transfusion. There are also reports of patients with deficiencies of haptoglobin and various complement components, such as C4a (Rogers antigen) or C4b (Chido antigen), developing anaphylactic reactions to platelets.

Transfusion-related acute lung injury

TRALI is a potentially life-threatening reaction that in many cases appears to be caused by passive transfusion of donor antigranulocyte antibodies (anti-HLA or anti-HNA antibodies), cytokines, biologically active lipids, or other substances. The resulting clinical picture is acute lung injury with noncardiogenic pulmonary edema with dyspnea, hypoxemia, hypotension, fever, and a chest x-ray showing bilateral infiltrates with pulmonary edema. Aggressive pulmonary support, including intubation, frequently is needed. Approximately 80% of patients improve within 48 to 96 hours, and 100% of patients require oxygen support with approximately 70% requiring mechanical ventilation. Infrequently, antibodies in the recipient may react with donor granulocytes that are introduced by units of RBCs or platelets. In some cases of TRALI, neither recipient nor donor-derived antibodies can be

identified. Other mechanisms have been advanced, such as the priming of neutrophils by bioactive lipids that accumulate during blood storage.

In 2007, TRALI represented approximately 65% of all transfusion-related fatalities reported to the U.S. FDA. Although TRALI accounted for only 38% of transfusion-related fatalities reported to the FDA in the 5-year period from 2011 to 2015, most likely due to widespread implementation of TRALI risk reduction strategies, it remains the leading cause of death due to transfusion in the United States.

The true incidence rate of TRALI is unknown, but it may occur in as many as 1 in 5,000 transfusions of any plasma-containing blood product (ie, RBCs, platelet concentrates, platelet apheresis units, and plasma), with a 5% to 10% fatality rate. TRALI can be difficult to distinguish from the manifestations of a patient's underlying medical problems, particularly those of cardiac origin, such as congestive heart failure and fluid overload from the transfusion. A consensus definition of TRALI is: acute lung injury (ALI) occurring during a transfusion or within 6 hours of completing a transfusion with no other temporally associated causes of ALI. ALI is defined as a syndrome of: (1) acute onset; (2) hypoxemia ($\text{PaO}_2/\text{FiO}_2 < 300 \text{ mm Hg}$, O_2 saturation $< 90\%$ on room air, or other clinical evidence); (3) bilateral pulmonary infiltrates, and (4) no evidence of circulatory overload. Clinical management is supportive with the goal of reversing progressive hypoxemia.

There is no universal method to prevent TRALI. Once blood from a particular donor is implicated in a case of TRALI, that donor is excluded from the donor pool. Preventing the first cases of TRALI by those donors, however, requires the elimination of all blood donors whose plasma contains anti-HLA or antineutrophil antibodies. For plasma, this is achieved by excluding female donors from the plasma donor pool because multiparous females are the most likely among a healthy donor population to have anti-HLA antibodies as a result of sensitization during pregnancy. When this approach was adopted in the United Kingdom in late 2003, where 60% of TRALI cases previously had been caused by plasma transfusions, no reports of TRALI deaths due to plasma occurred after 2004 (6 deaths occurred in 2005, none from plasma). Because platelets are chronically in short supply, excluding all female platelet donors is generally not feasible. Nevertheless, major blood suppliers in the United States now limit the collection of female platelets and/or screen for HLA/HNA antibodies in multiparous donors. Even with these precautions in place, cases of TRALI in which HLA or other granulocyte-specific antibodies do not appear to be responsible are not eliminated. Therefore, strict transfu-

sion criteria for plasma-rich blood products, early recognition, and prompt clinical management are key to managing TRALI risk. Reporting suspected cases of TRALI to the blood bank is also important in limiting potential risk to other patients by quarantine of any co-components from the same donation and evaluating the donor with possible exclusion from future donation if TRALI is confirmed.

Transfusion-associated circulatory overload (TACO)

Dyspnea with or without hypoxia during or after transfusion, accompanied by signs of volume overload—such as an increase in blood pressure, jugular venous distention, and elevated pulmonary arterial wedge pressure—represents transfusion-associated circulatory overload (TACO). At initial presentation, TACO and TRALI may be difficult to distinguish from each other. Despite increased awareness of TACO, it remains significantly underdiagnosed or at least underreported to hospital blood banks as a transfusion reaction. Despite this underreporting, TACO accounted for 24% of transfusion-related fatalities reported to the FDA between 2011 and 2015, making it the second most common cause of reported death due to transfusion in this time period (after TRALI). Risk factors for TACO include extremes of age, history of cardiac disease, renal failure, and transfusion of multiple blood components within a short period of time. An elevated brain natriuretic peptide may be helpful to distinguish TACO from TRALI in some cases. Therapy consists of diuretics and decreased blood administration rate.

Transfusion-associated graft-versus-host disease

TA-GVHD is an important risk in patients undergoing treatment of hematologic malignancies, patients undergoing HSCT, and patients with congenital immunodeficiency syndromes. The pathophysiology of TA-GVHD involves engraftment of small numbers of donor-derived passenger leukocytes into a host whose immune system is unable to recognize these cells as foreign and/or unable to eliminate them. Unlike HPSC transplantation-associated GVHD, in which the hematopoietic organ is donor derived and thus relatively protected from immune assault by donor-derived T cells, in *transfusion*-associated GVHD, the hematopoietic organ is recipient derived. Therefore, when TA-GVHD develops, mortality approaches 100% as a result of complications of severe pancytopenia. Patients may develop signs and symptoms of classic transplantation-associated GVHD, including skin rash, diarrhea, liver function test abnormalities, and other symptoms related to pancytopenia such as infection and bleeding. The infusion of any cellular blood product can theoretically cause

TA-GVHD. Irradiation of all cellular blood products before transfusion—but not conventional leukoreduction—virtually eliminates the risk of TA-GVHD.

TA-GVHD also has been described in immunocompetent patients when the donor is homozygous for an HLA haplotype shared with the recipient. Transfusion within relatively less HLA-diverse populations, such as in Japan, appears to increase the risk of TA-GVHD because of the increased prevalence of donors who are homozygous for an HLA haplotype shared with the recipient. This sets up a unidirectional HLA mismatch in which the recipient immune system is unable to recognize the donor-derived passenger leukocytes as being foreign and thus is unable to eliminate the passenger leukocytes; whereas the passenger T lymphocytes recognize the nonshared HLA allele on the recipient's cells and initiate a graft-versus-host reaction. For similar reasons, directed-donor transfusions between blood relatives, such as siblings or mother to neonate, increase the risk of TA-GVHD. Therefore, all directed donations of cellular blood products from blood relatives must be irradiated.

Infectious complications

Bacterial and parasitic transmission by transfusion

Bacterial contamination of platelet products is a significant issue given that platelets are stored at room temperature. Before the introduction of specific precautions to reduce bacterial contamination of platelet products, as many as 1 in 1,000 to 1 in 2,000 platelet units were contaminated with bacteria, resulting in clinical sepsis after 1 in 4,000 platelet transfusions. As bacterial contamination of platelets due to an infectious source became recognized as the most common cause of transfusion-associated morbidity and mortality in the United States (greater than hepatitis, HIV, and other viral sources combined), methods to limit and detect the presence of bacteria in platelet components were mandated.

Since the introduction of bacterial screening, the risk of septic transfusion reactions for apheresis platelets has declined to approximately 1 in 75,000, and the risk of a fatal septic reaction has declined to approximately 1 in 500,000. Efforts to limit the introduction of bacteria into platelets include the diversion of the first aliquot of donor blood from the collection bag to remove the skin core that otherwise would be introduced by the phlebotomy needle. Practices to detect the presence of bacteria in platelet units before dispensing to a patient include incubating an aliquot of the unit in a culture system and using a rapid strip immunoassay for bacterial antigens.

While platelet products are typically contaminated by gram-positive cocci, such as coagulase-negative staphylococci, sepsis associated with transfusion of RBC units is most often due to gram-negative organisms, particularly *Yersinia enterocolitica*. Fatal reactions to RBCs caused by contamination with *Yersinia enterocolitica* have been reported. This gram-negative organism can survive during refrigerated storage and lead to bacteremia or septic shock.

Malarial transmission by transfusion is uncommon, but cases are occasionally reported. Currently, no FDA-approved test is available to screen donors for malaria, and therefore screening is accomplished by donor questioning. Donors with a history of residence in a malaria-endemic area or travel associated with a risk of malarial exposure are deferred for up to 3 years, depending on the exposure.

With the immigration of individuals from South America to the United States, there is concern that Chagas disease may emerge as a common transfusion-transmitted infection. *Trypanosoma cruzi* parasites can survive several weeks of storage in blood, and contamination of blood products with this organism is already a significant problem in parts of South America. An FDA-approved blood donor-screening test for antibodies to *T. cruzi* is available. Blood donors need to be tested only at their first donation.

Transfusion-transmitted babesiosis has been reported in New England and the upper Midwest and has been identified in patients receiving platelets, refrigerated RBCs, and even frozen-thawed RBCs. Implementation of investigational tests is being evaluated for donor screening in areas where *Babesia* is endemic.

Borrelia burgdorferi, the etiologic agent of Lyme disease, has yet to be confirmed as having been transmitted by blood transfusions.

Viral hepatitis

Despite the exclusive use of volunteer blood donors and screening of donor blood for hepatitis B and hepatitis C viruses, posttransfusion hepatitis occasionally still develops due to blood donations during the brief initial period (~1 to 4 weeks) of viremia after exposure with a negative nucleic acid test. Acute transfusion-related hepatitis C virus infection is subclinical and anicteric in most cases.

With current anti-hepatitis C virus antibody tests and nucleic acid testing, it is estimated that the risk of post-transfusion hepatitis C is 1 per 1.1 million units transfused. The risk of HBV transmission by transfusion decreased from 1:220,000 to approximately 1:750,000 after implementation of HBV DNA testing. Table 13-6 summa-

Table 13-6 Infectious complications of transfusion

Infectious agent	Approximate risk per transfused unit
Hepatitis B virus	1:7.5 million
Hepatitis C virus	1:12.6 million
HIV-1, HIV-2	1:21.4 million
HTLV-1, HTLV-2	1:2.7 million
Bacterial sepsis	1:75,000 (platelet transfusion); 1:250,000 to 1:10 million (RBC transfusion)

rizes the estimated risks of various transfusion-associated infections.

Photochemical pathogen inactivation strategies appear to be both efficacious and relatively sparing in terms of qualitative platelet function, although posttransfusion platelet increments may be slightly smaller.

HIV and human T-cell lymphotropic viruses

The risk of acquiring HIV-1 or HIV-2 infection as a result of transfusion currently is estimated to be 1 in 1.5 million. Nucleic acid amplification testing for HIV has reduced the window of serologic conversion from 16 days to about 9 days.

Human T-cell lymphotropic virus 1 (HTLV-1) is a retrovirus associated with adult T-cell leukemia or lymphoma and tropical spastic paraparesis. Because asymptomatic blood donors can transmit this virus, screening for HTLV-1 in blood donors is currently performed in the United States. Several cases of neuropathy had been reported in transfusion recipients before the availability of testing. HTLV-2, a related virus with antigenic cross-reactivity to HTLV-1, is endemic in certain Native American populations and also has been found in a high proportion of intravenous drug users. The risk of HTLV transmission by transfusion using current test methods is approximately 1 in 2.7 million.

West Nile virus

During the 2002 West Nile virus (WNV) epidemic in the United States, 23 individuals acquired WNV after blood transfusion, developing fever, confusion, and encephalitis characteristic of WNV infection within days to weeks of transfusion. As a result, blood centers now use nucleic acid-based testing to screen all donations for WNV. In a survey of 2.5 million donations in 2003, 601 donations (0.02%) were found to contain WNV. A subsequent follow-up study detected no cases of transfusion-transmitted WNV infection among recipients of tested blood; however, rare breakthrough transmissions have been reported.

Parvovirus B19

Rare transmissions of parvovirus B19 by transfusion have been recognized. A recent study documented persistence of low levels of parvovirus B19 DNA in a high percentage of multitransfused patients. The long-term clinical implications of this finding currently are unknown. Parvovirus (and other viruses without a lipid envelope, such as hepatitis A virus) is not eliminated by solvent detergent treatment. Acute parvovirus B19 infection can result in impaired erythropoiesis and cause an aplastic crisis in patients with SCD and other hemolytic diseases. Infection with this virus can also result in significant fetal harm when a pregnant woman is infected during weeks 9 to 20 of pregnancy. There is no currently available blood donor screening assay for this virus.

Cytomegalovirus

Leukocytes are invariably present in RBC and platelet products, even after leukoreduction, and they are capable of transmitting CMV infection. Transfusion-transmitted CMV infection is an important issue in transfusion of cellular blood products to neonates, particularly low-birth-weight infants born to seronegative mothers, HSCT recipients, and other highly immunosuppressed patients. The risk of acquiring CMV from transfusions is particularly high when pretransplantation serologic testing reveals that neither the HPSC donor nor the recipient previously has been exposed to CMV. In addition, transplantation recipients are at increased risk for transplantation-associated CMV reactivation when either the donor or the recipient is seropositive for CMV before transplantation. The latter consideration often affects the choice of HPSC donors.

For these reasons, some institutions use blood products obtained exclusively from CMV-seronegative donors when providing blood products to neonatal recipients or recipients of HPSC transplantations. However, randomized comparison of leukoreduced vs CMV-seronegative blood components in CMV-seronegative HSCT recipients (with seronegative donors) found no significant difference in the incidence of CMV infection, and CMV disease as a composite outcome. Thus, most transplantation centers use prestorage leukoreduced blood components for CMV prevention. Other institutions simply use leukoreduced blood products in all recipients, regardless of CMV status. The latter strategy has the additional advantage of reducing the risk of alloimmunization to HLA antigens and subsequent refractoriness to platelet transfusions.

Blood management

CLINICAL CASE

A 44-year-old multiparous female requires orthopedic surgery. Pretransfusion testing reveals antibodies to 3 RBC antigens: K (Kell system), Fy^a (Duffy system), and E (Rh system). Crossmatch-compatible blood is transfused, and the patient does well. A second operation is needed, and at this time repeat screening of the patient's plasma detects an additional antibody directed against c (Rh system). Because of the multiple antibodies, a large number of donor units must be screened to find the required number of antigen-negative units. The hematologist advises the surgeon that a comprehensive blood management approach should be considered to reduce the need for further allogeneic transfusion in this patient.

The concept of blood management has been steadily gaining in popularity with the recognition of the high costs associated with transfusion, high frequency of inappropriate utilization of blood products, and an increasing range of adverse effects potentially associated with transfusion. Avoidance of unnecessary allogeneic transfusion is the ultimate goal of blood management, and a multidisciplinary approach is required to achieve it. The elements of blood management include decreasing the need for transfusion, using the patient's own blood when possible, optimizing utilization of allogeneic blood products when transfusion is indicated, and performing utilization reviews with auditing and benchmarking to initiate and maintain the behavioral changes required for the broad application of blood management in a hospital setting.

Iatrogenic and preoperative anemia

The cornerstone of decreasing the need for transfusion is appropriate medical management of anemia, particularly in the preoperative setting in which anemia is the most important predictor of perioperative transfusion. Management of preoperative anemia often can be achieved simply through iron replacement; use of erythropoietin may be indicated in some cases. Avoidance of iatrogenic anemia by avoiding unnecessary blood draws is equally important. In the setting of ICUs, routine blood draws have been demonstrated to result in the loss of the equivalent of 1 to 2 units of RBCs per week. All blood tests ordered should be justified and actively contribute to clinical decision making. The frequency, timing, and volumes of blood draws, including use of lower-volume blood collection tubes when appropriate, should be coordinated to limit the volume of patient blood collected.

In the past, preoperative autologous donation, where the patient would donate blood for his or her own use in the weeks before surgery, used to be the most common approach to avoid allogeneic transfusion for elective surgical cases. Although the use of autologous blood may eliminate transfusion risks because of transfusion-transmitted infection (except for bacterial contamination of the unit), the risk of transfusion of ABO-incompatible blood due to a clerical error still exists (ie, the inadvertent transfusion of the wrong patient's autologous blood). Likewise, transfusion-associated complications such as those related to fluid overload in a patient with cardiac disease can occur. Therefore, unless the clinical condition of the patient actually warrants transfusion, autologous units of blood should not be used simply because they are available and "won't hurt." Use of preoperative autologous blood donation is now broadly discouraged, as approximately 50% of autologous units are never transfused and patients who donate autologous units preoperatively may present to surgery with anemia that increases their overall risk of transfusion, particularly if the interval between donation and surgery is short. Of note, directed donations from relatives or friends selected by the patient have not been shown to decrease transmission of infectious agents compared to units from the general blood supply. In fact, blood from first-degree relatives must be irradiated to prevent TA-GVHD, and family donors can HLA alloimmunize the patient through transfusion, which may preclude a subsequent transplant. Directed donors are also more likely to be first-time donors, who have a higher incidence of infectious disease positivity. For these reasons, directed donations are generally discouraged.

Intraoperative techniques

A number of surgical, anesthetic, and pharmacological approaches can be utilized to reduce intraoperative bleeding. Use of the patient's own blood to minimize the need for, or entirely avoid, allogeneic transfusion may be accomplished through acute normovolemic hemodilution (ANH) and RBC salvage or perioperative autotransfusion. ANH involves removal of 1 or more units of whole blood in the operating room immediately before surgery, with adequate fluid replacement to maintain an iso- or normovolemic state. Blood shed during surgery is dilute in this case, theoretically resulting in a lower net loss of RBC mass after return of the whole blood units to the patient toward the end of the case. The units collected by ANH may have the added benefit of providing additional platelets and coagulation factors. ANH has not been established definitively to avoid allogeneic transfusion. Conversely, intraoperative cell salvage can significantly reduce

the need for allogeneic transfusion, particularly in cases associated with high-volume blood loss. In this approach, blood is suctioned from the operative field into an anti-coagulated reservoir and then washed with normal saline. The washed salvaged RBCs are concentrated for reinfusion into the patient. When using cell salvage techniques, precautions must be taken to avoid potential hazards, such as air emboli and infusion of inadequately washed products. In some cases, postoperative wound drainage may be collected, filtered, and administered with or without washing. Many Jehovah's Witnesses consent to autologous transfusion using cell salvage, potentially allowing more complex surgeries to be performed in this patient population. The technique can also be helpful in patients for whom it is difficult to find compatible blood because of the presence of multiple RBC antibodies.

Judicious transfusion

Care should always be taken to transfuse the smallest amount of blood products required to achieve the desired outcome. It is unnecessary to correct a cytopenia or a clotting factor deficiency to normal levels; transfusion should be directed toward restoring only functionally adequate levels. For example, many patients with chronic anemia or thrombocytopenia tolerate much lower blood counts than patients with acute cytopenias involving the same lineages, and most patients tolerate clotting factor levels below 50% without difficulty. One of the major behavioral changes incorporated into most blood management programs is a shift in practice from transfusing RBC units in multiples to a strategy of single-unit transfusions with subsequent reassessment of patient status and need for further transfusion.

Auditing of compliance with institutional transfusion guidelines, internal and external benchmarking, and ongoing data-driven process improvement projects all contribute to improved blood product utilization and systematic application of blood management concepts. The institutional oversight for such activities usually is provided by hospital transfusion committees, which typically include broad multidisciplinary representation from transfusion medicine, hematology, anesthesiology, surgery, internal medicine, nursing, pharmacy, laboratory medicine, and hospital administration.

Bloodless medicine

Currently, no licensed blood substitutes are available for clinical use in the United States. A recent meta-analysis of hemoglobin-based blood substitutes found excess myocardial infarction and mortality in surgical patients who received the blood substitute compared with controls;

patient groups included trauma, cardiac surgery, vascular surgery, and elective orthopedic surgery.

Erythropoiesis-stimulating agents can be used in patients who decline transfusion, either therapeutically to treat anemia or prophylactically before elective surgery. The management of Jehovah's Witness patients who require chemotherapy for hematologic malignancies or HSCT can be challenging. A comprehensive approach is required, including reduced-intensity conditioning chemotherapy, reduced phlebotomy and gastrointestinal blood loss, optimized pretransplantation blood counts using iron and folate, erythropoiesis-stimulating agents, and possibly thrombopoietin mimetic agents, as well as prophylactic use of antifibrinolytic agents during the period of thrombocytopenia.

KEY POINTS

- Avoiding iatrogenic anemia can help reduce the need for allogeneic transfusion in all patient populations.
- Transfusion only when indicated (right product to the right patient at the right time and for the right reason) can help avoid unnecessary risks.
- Preoperative medical management of anemia before elective surgery can reduce perioperative transfusions.
- Preoperative autologous donation generally is discouraged due to wastage of collected units, the residual risks of clerical error, bacterial contamination, and volume overload, as well as the preoperative anemia associated with these donations.

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14

Cellular basis of hematopoiesis and stem cell transplantation

IRUM KHAN AND KIM-HIEN T. DAO

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Introduction and historical perspective

Humans produce approximately 300 billion blood cells per day. Hematopoiesis is the process by which blood cells are made by transiting through a hierarchy of hematopoietic stem and progenitor cells (HSPCs). Hematopoietic stem cells (HSCs) are defined by their ability to self-renew as well as differentiate into the progenitors that replenish the entire blood system. Residing at the top of this hierarchy, HSCs are located in a number of embryonic niches, settling in the bone marrow (BM) in adult life. Contrary to previous assumptions, HSCs are a heterogeneous cell population, and likely number tens of thousands of cells in adult life, giving rise to hundreds of millions of hierarchically organized highly heterogeneous progenitor cells, which in turn differentiate into precursor cells and eventually mature effector cells. Although the field of stem/progenitor cell biology has grown dramatically over the past decades, bone marrow transplantation (BMT) has been in routine clinical practice for >50 years and is the only routine, widely used example of stem/progenitor cell therapy. Identification of BM HSPCs emerged after it was recognized that survivors from atomic bomb explosions in 1945 died of hematopoietic failure from radiation damage. In the early 1960s, a series of seminal experiments by Till and McCulloch showed that the transfer of BM cells from donor mice into lethally irradiated host mice resulted in the formation of macroscopic colonies (called spleen colony-forming unit [CFU-S] cells) of myeloid, erythroid, and megakaryocytic cells in the spleens of the recipients. These colonies arise from a single implanted cell and were the first demonstration of the existence of a repopulating hematopoietic cell that could also differentiate. This chapter summarizes the development, differentiation, and localization of HSCs and the hematopoietic niche, as well as aspects of HSC purification and expansion for transplantation.

Hematopoiesis through development and into adult life

Hematopoiesis occurs in waves and at multiple discrete anatomical sites that change through development (Figure 14-1). In humans, like other vertebrates, the initial wave of hematopoiesis occurs in the extraembryonic yolk sac (YS) blood islands from weeks 3 to 6 of gestation. The YS primarily produces primitive erythroid cells (termed *primitive erythropoiesis*), expressing embryonic globins that

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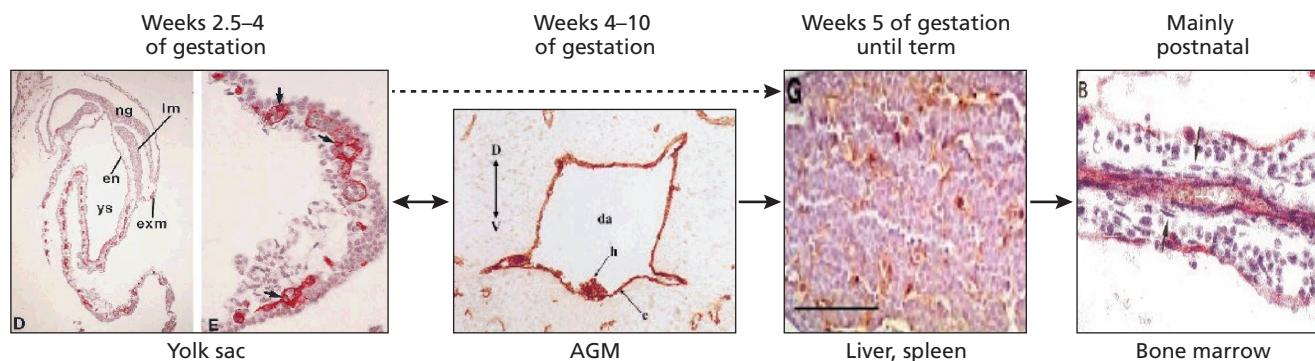


Figure 14-1 Changing anatomical locations of hematopoiesis through development. Hematopoiesis is initially detected in the extra embryonic yolk sac, then in the embryo in a region known as the aorta-gonad-mesonephros (AGM), the placenta, the umbilical arteries, and vitelline vessels. It then shifts to the fetal liver and finally to the bone marrow.

deliver oxygen to tissues in the rapidly growing embryo. Additional studies suggest that primitive hematopoiesis produces myeloid and lymphoid cells (eg, macrophages and natural killer [NK] cells). Interestingly, the developmental potential of embryonic hematopoiesis closely resembles hematopoietic cells derived from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs).

Primitive hematopoiesis is transient and replaced by adult or definitive hematopoiesis that sustains blood production throughout development and postnatal life. Embryonic hematopoietic activity is detected at 4 to 5 weeks of gestation in a region around the ventral wall of the dorsal aorta, called the aorta-gonad mesonephros (AGM). In early development, blood cells arise in close connection with the vascular structures (both in the YS and the dorsal aorta), giving rise to the notion that there may be a common precursor cell population that produces both blood and blood vessel cells called a hemangioblast, or that hematopoiesis arises directly from specialized “hemogenic” endothelial cells, such as those lining the ventral aspect of the dorsal aorta. In mice and other animals, studies have shown that definitive hematopoietic stem cells with serially transplantable activity, together with long-term engraftment capacity, are found in the AGM. It is still a matter of much debate whether HSCs arise from the embryo proper, from the AGM, or by colonization from the YS.

HSCs are then detected in the developing fetal liver, spleen, and thymus, from 6 to 22 weeks in humans, where they expand and differentiate into committed progenitor cells. Expansion and differentiation of HSCs allows for development of definitive red cells, myeloid cells, and lymphoid cells (T cells that develop in the thymus and B cells in the marrow). HSCs are then later detected in the BM. It is also unclear if HSCs from the AGM migrate and colonize the other embryonic sites or whether they arise

de novo at these other sites. It is not clear whether HSCs must first reside in the fetal liver before seeding the BM. A large transient pool of HSCs has been identified in the placenta of mice around the time of AGM HSC development. It remains to be determined whether an equivalent population of HSCs exists in developing human placenta. Given that HSCs isolated from different locations or cell sources (eg, BM, fetal liver, placenta, hESCs/iPSCs) and from organisms of different ages have been shown to have distinct gene expression patterns and phenotypic features, this may have implications regarding choice of stem cell sources for human transplantation therapies.

Murine HSCs are heterogeneous with respect to lineage output—generating megakaryocyte, myeloid or lymphoid progenitors, or balanced progenitor output. Quantitation of HSC output in mice has been more technically feasible than studies in humans. Using limiting dilution analysis of various tissues, a quantitative and temporal relationship has been modeled in developing mice. AGM and YS HSCs enter the circulation and provide a pool of HSCs recruited to the liver. In whole BM cells, the estimated frequency of HSCs, defined by functional ability for long-term engraftment and multilineage reconstitution, is 3 per 100,000 cells. In the mouse, fetal HSCs show extensive proliferation and tend toward greater lymphoid output. During adult life, HSCs are more quiescent. Functional HSCs are reduced with age, and lymphocyte-producing HSCs diminish relative to myeloid-biased HSCs, potentially contributing to immune alterations observed with aging. These studies also demonstrate that changes in functional potential of HSCs during development and aging correlate with changes in gene expression and growth factor requirements. Additionally, HSCs demonstrate epigenetic changes over time. Similar detailed studies in humans still need to be performed, but the murine studies raise the

prospect that there will be similar changes in the functional potential of human HSPCs and that this may in part be the cause of the relative lymphoid deficiency in the aging population. In humans, the discovery that mutations in genes encoding epigenetic regulators are relatively common with aging, and affect the same genes recurrently mutated in myeloid malignancies, further demonstrates the importance of epigenetic control of normal hematopoiesis.

Somatic mutation(s) in hematopoietic cells (also known as clonal hematopoiesis) in the absence of clinical or laboratory evidence of hematologic malignancy has been recently described in great detail and occurs at relatively high frequency in an aging population (~10% to 30%) and in patients who received cytotoxic chemotherapy for lymphoma (~30%). The most common genes involved in the aging population include *ASXL1*, *DNMT3A*, and *TET2*, with the additional enrichment of *PPM1D* and *TP53* genes in the cytotoxic chemotherapy-exposed population. The somatic mutations generally lead to loss-of-function of 1 allele. A high variant allele frequency, a marker of the clonal hematopoietic cell burden in the blood, correlates with outcomes including increased deaths related to cardiovascular disease (strokes and heart attacks) and increased incidence of hematologic malignancies or therapy-related myeloid neoplasms. The biological connection between mutant hematopoietic cells and cardiovascular disease is felt to be in part related to unchecked inflammation, which has been studied in *TET2*-deficient mouse models. Interestingly, in a nonhematologic cancer patient population, therapy-related clonal hematopoiesis was associated with increased deaths due to progression of the primary nonhematologic cancer. In addition to unchecked inflammation playing an important role in tumor microenvironment and tumor progression, the role of impaired immunogenic cancer surveillance and cell death should be further explored.

Hematopoiesis: a hierarchical differentiation cascade

The hematopoietic stem and progenitor cells are highly heterogeneous populations defined by 2 key properties: variable ability to self-renew and variable ability to differentiate and generate mature blood cells. At the top of the hierarchy are HSCs. They have extensive self-renewal capability and differentiate into all blood cell types (Figure 14-2). During normal steady-state hematopoiesis, adult HSCs cycle slowly and are relatively resistant to cytokine stimulation. Within the HSC compartment, long-term stem cells are usually quiescent and rarely divide. Short-term stem cells are more proliferative. The remarkable ability of HSCs, at the single-cell level, to reconstitute and maintain a functional hematopoietic system over extended periods of time *in vivo* demonstrates these key properties.

Self-renewal allows HSCs to be transplanted between individuals, and the surviving HSCs engraft, proliferate, and differentiate for the life of the recipient. HSCs can be serially transplanted for many generations between recipients. The most primitive HSCs are rare, representing approximately 1 in 10^4 to 10^6 BM cells based on experimental models.

When HSCs are recruited into active hematopoiesis, they exit the G0 phase of the cell cycle and undergo mitosis leading to daughter cells with either identical cell fate (symmetric cell division) or different cell fate (asymmetric cell division). In the case of symmetric cell division, the 2 daughters can both retain HSC functions, or both display an activated differentiation program. In the case of asymmetric cell division, 1 daughter cell is a replicate of the parent cell (self-renewal) and 1 daughter cell displays an activated differentiation program. This distinctive, asymmetric division process is the basis for long-term preservation of HSCs while enabling continued production of mature cells. The daughter cells that undergo differentiation proceed through a series of maturational cell divisions, culminating in the generation of progenitor cells.

Progenitor cells are also hierarchically arranged. As they differentiate from stem cells and through the progenitor hierarchy, they progressively lose self-renewal and become restricted in their differentiation potential such that more multipotent progenitors give rise to oligopotent and finally monopotent progenitors. Progenitors are highly proliferative and very cytokine responsive. New studies of murine hematopoiesis suggest that in contrast to transplantation, the source of most of the cells produced daily by the blood system during normal steady-state blood production is maintained by the continuing expansion of thousands of hematopoietic progenitor cells, each with a minimal contribution to mature progeny. Single-cell transplant studies in mice have also revealed a bypass pathway that produces long-term repopulating myeloid progenitors. This pathway may be operative under stress, as progenitor populations most readily respond to stress conditions in order to up- and downmodulate production of specific blood cell types. Progenitors differentiate into lineage-restricted precursor cells and eventually mature effector cells of the hematopoietic system. These mature lineages include erythroid cells for oxygen transport, myeloid and lymphoid cells that provide immune defense, and megakaryocytes and platelets essential for hemostasis.

Finally, our current view of the hematopoietic cellular hierarchy is changing based on data generated using newer technologies such as more advanced lineage tracing and single-cell omics profiling. Some key conceptual changes include: (1) hematopoiesis is a continuous differentiation

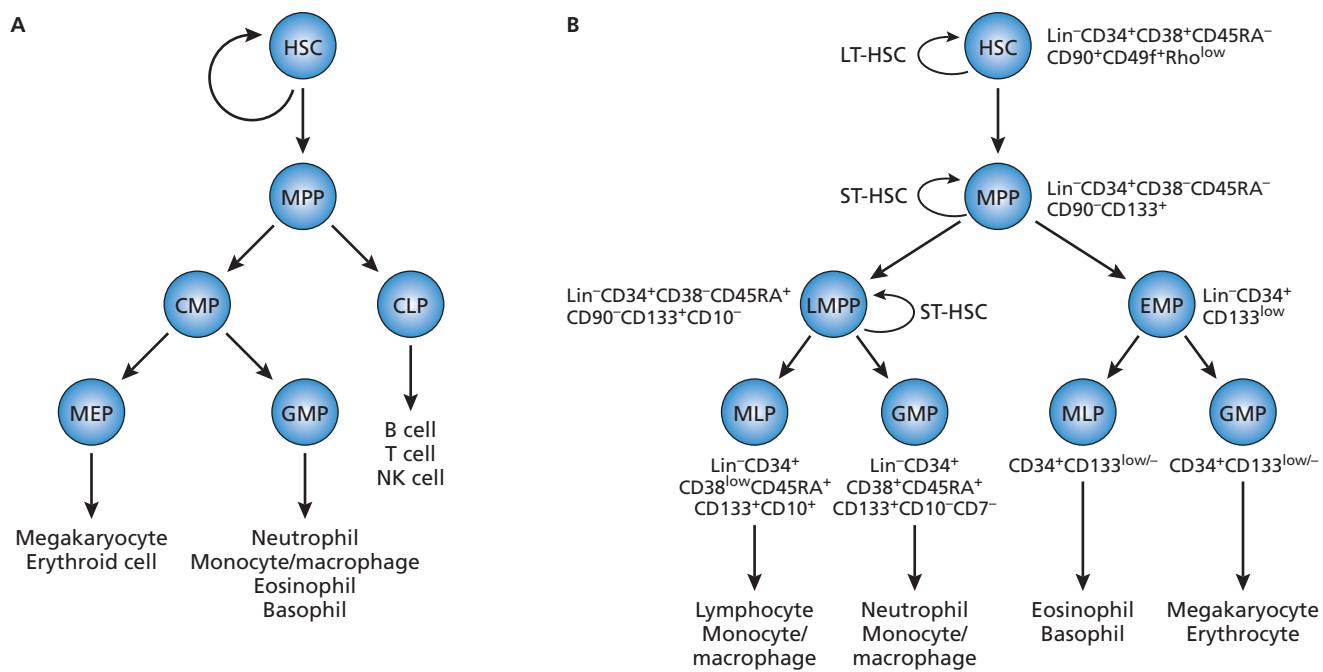


Figure 14-2 Alternative hierachal maps of human hematopoiesis. (A) In the classical model of human hematopoiesis, a self-renewing HSC gives rise to a multipotent progenitor (MPP) that bifurcates into a common myeloid progenitor (CMP) and a common lymphoid progenitor (CLP), which then eventually give rise to mature myeloid and lymphoid cells respectively. (B) Based on a compilation of more recent data, the MPP appears to give rise to an LMPP, both of which may have short-term self-renewal capacity, and an erythromyeloid progenitor (EMP), differentiated by CD133 expression. The hierarchy following the LMPP further bifurcates into a multilymphoid progenitor (MLP) and a granulocyte macrophage precursor (GMP). The EMP gives rise to a precursor that produces eosinophils and basophils (EoBP) and a megakaryocyte/erythroid progenitor (MEP). It is highly likely that as our ability to functionally and molecularly interrogate HSPCs at a single cell level improves, our understanding of the hierarchy will be further refined. Surface antigen phenotypes of human hematopoietic stem and progenitor cells are indicated. The immunophenotypes of the EMP, EoBP and MEP are not as well characterized as the other progenitors. LT-HSC, long-term HSC; ST-HSC, short-term HSC.

process and not a stepwise process with discrete functional and phenotypic markers; (2) lineage segregation occurs earlier in the hierarchy at the HSC level rather than at the progenitor level; (3) hematopoiesis is not lineage-balanced and HSCs are not homogenous and can be affected by aging, stress, and injury. Our understanding of the hierarchical relationships between populations, the plasticity of commitment and the nature of the functional potential of populations, will increase as we better purify HSPC populations using newer technologies.

Phenotypic characterization and isolation of HSPCs

Attempts to purify stem cell populations have used a combination of approaches based on physical and biologic properties and cell surface marker expression of HSPCs. Early work on murine BM revealed that the transplantable HSCs copurified with lymphocytes and led to the idea that HSCs are morphologically indistinguishable from lymphocytes. Density gradient separation, such as Ficoll and Percoll gradient, are commonly used as a pre-enrichment step

in stem cell purification protocols. Progenitor cells cycle actively, whereas HSCs are relatively quiescent. This difference has been exploited in techniques for HSC enrichment in mouse and human systems. Treatment of mice with the antimetabolite agent fluorouracil markedly reduces progenitor cells, while relatively sparing populations enriched in HSC activity. More recently, considerable progress has been made prospectively isolating HSPCs using flow cytometry and cell surface markers. However, it is difficult to compare different immunophenotyping strategies with respect to quantifying the purity of the HSC population, as various factors such as source of HSCs (umbilical cord vs marrow), route of transplant (intravenous vs intrafemoral), the type of immunodeficient mouse used for xenografting, and pretransplant manipulation of the cells can all affect the outcome.

Figure 14-3 schematically illustrates how HSCs and progenitors are isolated and tested for function. Hematopoietic tissues are isolated then disassociated through mechanical or enzymatic disruption, and then labeled

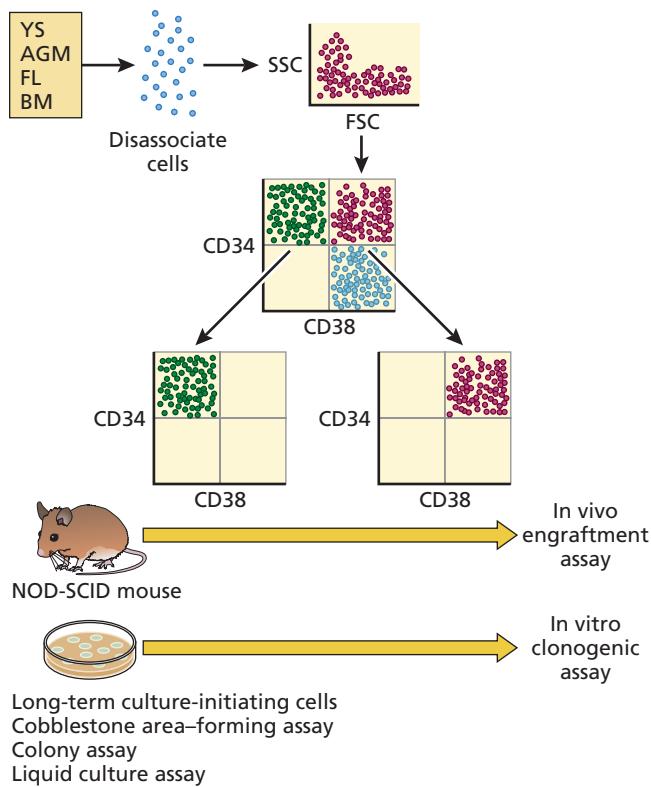


Figure 14-3 Isolation of HSCs. This figure shows how HSCs can be isolated from different sources. Cells are initially disassociated and stained with multiple antibodies. They are then analyzed and sorted with a fluorescence-activated cell sorter. Here mono-nuclear live cells are separated in gate 1. These live cells are then analyzed for CD34 and CD38 expression. Those live cells that are CD34⁺CD38⁻ are enriched for stem cell potential (green circles). Further purification can be undertaken on the basis of additional cell surface markers such as CD45RA, CD90, CD49f, and efflux of dyes (eg, rhodamine). To test the functionality of isolated (sorted) cells, cells can be tested in in vivo assays (transplanted into immunodeficient mice such as the NOD-SCID mouse model) and in vitro in long-term culture (long-term culture-initiating cell culture assay and cobblestone-area forming assay), clonogenic colony assays and liquid culture assays. FL, fetal liver; FSC, forward side scatter; SSC, side scatter. Redrawn with permission from Hoffbrand AV, Pettit JE, Vyas P. *Color Atlas of Clinical Hematology*. 4th ed. Philadelphia, PA: Mosby, Ltd; 2010. © John Wiley & Sons Ltd.

with panels of fluorescently conjugated antibodies. The cell populations can then be analyzed and separated on a fluorescence-activated cell sorting. Early studies to isolate human HSCs found that approximately 1% of human BM cells express CD34. Isolation of CD34⁺ cells enriches for HSPCs, which have hematopoietic engraftment potential when transplanted into irradiated nonhuman primates. Similarly, human CD34⁺-selected cells contain stem cells capable of fully reconstituting the lymphohematopoietic system in humans after myeloablative chemotherapy and

radiation therapy. Although it is clear that the CD34⁺-expressing population contains a long-term, repopulating HSC, there is some evidence of an upstream deeply quiescent CD34⁻ HSC that gives rise to the CD34⁺ HSC. Approximately 5% to 25% of CD34⁺ cells also express low to moderate levels of CD90. CD90 expression by human hematopoietic cells decreases with differentiation, and most lineage-restricted progenitors are CD34⁺CD90^{+/low} cells. Additional studies demonstrate that human HSCs do not express mature cell lineage markers (Lin⁻) or CD45RA or CD38. Isolation of Lin⁻CD34⁺CD38⁻CD45RA⁻CD90⁺ cells provides a relatively easy method to sort for putative HSCs based on in vitro and in vivo studies. However, this remains a heterogeneous population. Sorting for the integrin CD49 further enriches for HSCs. Others have combined some of these markers with ability of HSCs to efflux dyes (eg, the mitochondrial dye rhodamine 123). HSCs, but not progenitor cells, express high levels of the verapamil-sensitive multidrug-resistance membrane efflux pump (P-glycoprotein), which confers resistance to multiple chemotherapeutic agents. This pump also excludes certain fluorescent dyes, such as rhodamine 123 or Hoechst 33342. By using these dyes in combination with flow cytometry, it has been possible to identify a population of hematopoietic cells with low dye retention, so-called side population (SP) cells. Although this population is markedly enriched for HSCs, SP cells still represent a heterogeneous mix and are not equivalent to pure HSCs. Although the SP phenotype has been useful in characterizing HSCs (and possibly other non-HSCs) isolated from mice, this characteristic has not translated as easily into the human system.

Downstream of the long-term repopulating HSCs, one study has shown that CD133 expression differentiates various progenitor populations, such that high CD133 defines the lymphoid-primed multipotent progenitor (LMPP) that matures into lymphoid, monocyte/macrophage, and neutrophil lines. In contrast, CD133^{low/-} progenitors differentiate into megakaryocytes, erythrocytes, eosinophils, and basophils (Figure 14-2). In mice, the immunophenotype of c-Kit⁺, Thy-1⁺, Lin⁻ (a cocktail of surface markers found on mature cells of distinct lineages), and Sca-1⁺ (so-called KTLS cells) enriches for cells with HSC activity. Flk2 expression can be used to distinguish long-term repopulating HSCs (LT-HSCs; Flk2⁻) from short-term repopulating HSCs (ST-HSCs; Flk2⁺). Other protocols have used the signal lymphocyte activation molecule (SLAM) family receptors CD150, CD244, and CD48 to isolate murine HSCs that are highly purified as CD150⁺CD244⁻CD48⁻. The number of murine HSCs estimated using either the KTLS/FLK2⁻ or the SLAM immunophenotype

is ~10,000 HSCs/mouse. Finally, the SLAM phenotype does not translate for isolation of human HSCs.

Though current HSPC populations are still impure, they have allowed isolation of cell populations of defined functionality and are useful to identify genes and signaling pathways that mediate human HSPC differentiation. Isolation of distinct HSPC populations is also beginning to permit careful dissection of the hierarchical relationships between different blood cell populations. This is essential in describing the cellular basis of normal hematopoiesis. In turn, this is critical when trying to understand (1) the normal cellular compartments where genetic and epigenetic changes initially occur in hematological diseases (ie, the disease-initiating cell populations); (2) the cell compartments where subsequent mutations/epigenetic change is acquired during disease evolution and how this changes the hematopoietic hierarchy; and (3) the cell populations that propagate hematopoietic disease. Advances in cell sorting, genetic analysis, and other technologies are making analysis of HSCs increasingly precise. This progress certainly will provide additional insights into HSC biology and heterogeneity.

Stem/progenitor cell assays

A number of in vitro and in vivo assays have been developed to test HSPC function. It is useful to have background knowledge of them as the assays have defined HSPC populations.

Colony-forming assays

The identification of a cell capable of in vivo clonal differentiation by Till and McCulloch (1961)—for example, in spleen colonies (Figure 14-4A)—prompted other groups to develop a simple quantitative assay for the growth and differentiation of single-cell suspensions of mouse BM in vitro. When hematopoietic cells were cultured in a semi-solid medium (typically, soft agar or methylcellulose), discrete colonies were formed and included cells in multiple stages of differentiation (Figure 14-4B). In line with the properties observed for CFU-S, it subsequently was established that colonies generated in vitro could be initiated by the proliferation of a single colony-forming cell (CFC). In contrast to the self-renewal potential of most CFU-S, colonies grown in vitro displayed more limited ability to proliferate in secondary cultures. Therefore, CFCs were suggested to define a population of committed progenitors. That is why today we define progenitors as those cells that can form a colony in an in vitro colony-forming assay. HSCs can also form colonies. Alternatively, one cannot exclude the possibility that our current HSC popula-

tions are impure and contain more committed progenitor populations.

Long-term bone marrow culture

Attempts to develop procedures that mimic the marrow microenvironment resulted in the development of long-term BM cultures. In these assays, formation of an adherent stromal cell layer, which produces and deposits an extracellular matrix meshwork, is a prerequisite for the development and maintenance of hematopoietic cells. In association with the feeder layer, hematopoietic cells proliferate and differentiate over several months in culture-releasing clonogenic and mature cells. The ongoing production of these cells is the result of differentiation and proliferation of primitive cells. In recognition of their method of detection, these cells have been called long-term culture-initiating cells (LTC-ICs). They represent primitive immature hematopoietic cells that can be assayed in vitro. The presence of LTC-ICs can be detected by assaying for the presence of CFUs in cultures maintained for a minimum of 5 weeks. Beyond this point, any CFCs (progenitor cells with shorter survival time) initially present in the culture should have disappeared through differentiation or death, and those detected are the result of differentiation by LTC-IC. LTC-ICs are not necessarily true HSCs, and limits of these assays make it difficult to know whether these cells are capable of definitive long-term reconstitution and maintenance of hematopoiesis in vivo. In vivo studies can be expensive and cumbersome, however, and they come with their own caveats. Therefore, LTC-IC studies provide a reasonable in vitro surrogate assay for early human hematopoietic cells with similar functions to HSCs.

Transplantation assays

The definitive assay for mouse HSC activity is the ability to provide long-term (>4 months) repopulation of all blood lineages of myeloablated host mice. Human HSCs cannot be similarly identified, however, except in a clinical study. Therefore, xenograft models commonly have been used as another surrogate assay for human HSCs. This work originally involved transplanting human hematopoietic cells into severe combined immune-deficient (SCID) mice. However, more immunodeficient and/or radioresistant mouse strains such as NOD-SCID/IL-2R γ ^{-/-} (commonly termed NSG or NOG mice) or NOD/Rag1 $^{1-/-}$ /IL-2R γ ^{-/-} (NRG) or further engineered to express human cytokine or other molecules that aid cell survival (for example, IL-3, Steel factor, GM-CSF termed NSGS mice; human thrombopoietin or human SIRP1a) are now used for these analyses. Human cells giving long-

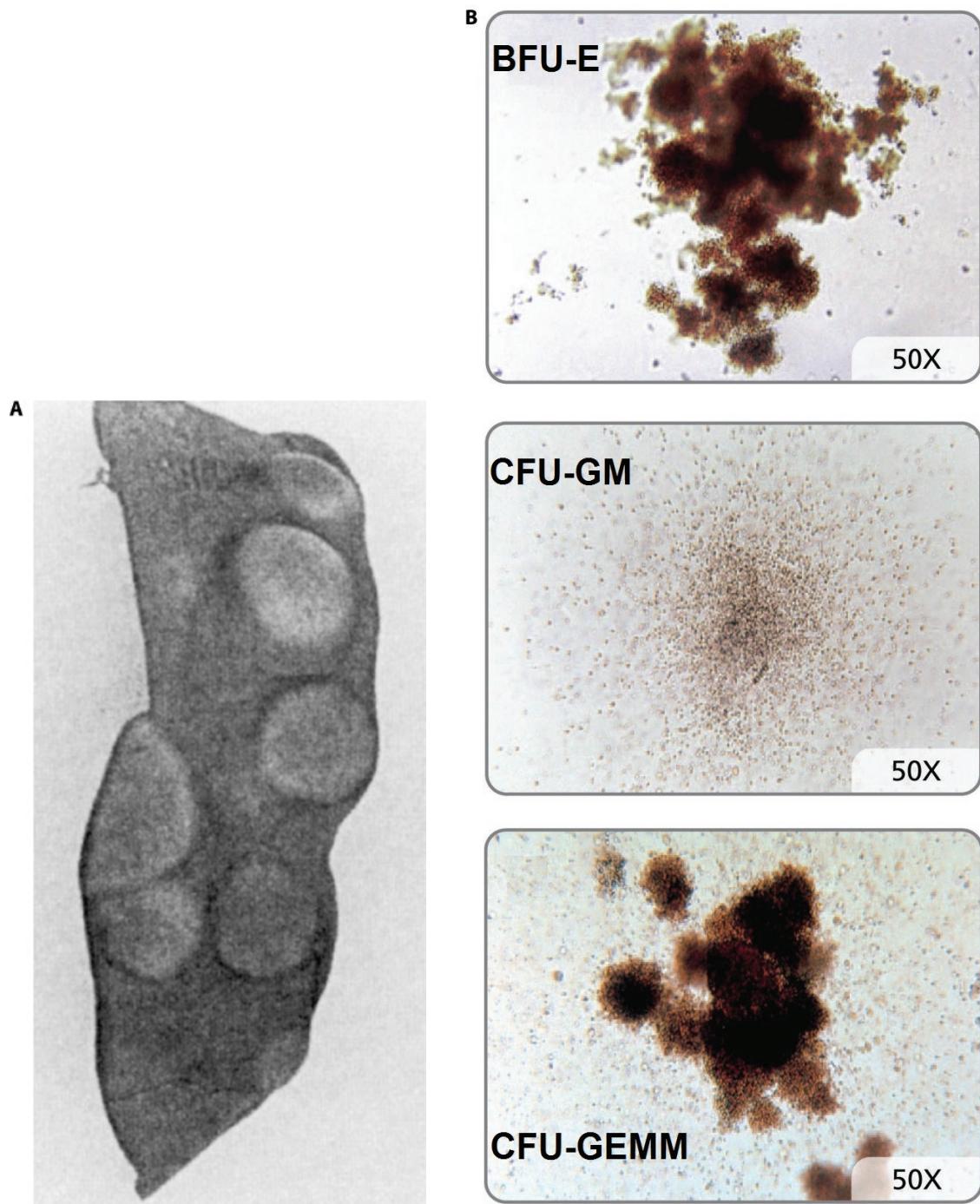


Figure 14-4 (A) Spleen colony-forming unit (CFU-S) assay. Macroscopic splenic hematopoietic colonies arising from the CFU-S stem/progenitor cell 14 days after injection of murine BM into lethally irradiated mice. Reproduced with permission from Williams, DA, Stem cell model of hematopoiesis. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Cohen HJ, eds. Hematology: Basic Principles and Practice. New York, NY: Churchill Livingstone, 1995. (B) Examples of colony-forming assays of human hematopoietic progenitor cells. These include burst-forming unit erythroid (BFU-E), CFU granulocyte/macrophage (CFU-GM), and CFU granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM). Reprinted with permission from STEMCELL Technologies (www.stemcell.com).

term engraftment in these immunodeficient models are considered more primitive and clearly distinct from prior multipotent primitive human hematopoietic cell populations identified using *in vitro* methodology. Other transplantation models have been developed; for example, using fetal sheep for xenografts or nonhuman primates for autologous transplantation studies (often using gene transfer into putative HSCs). Additionally, zebrafish (where HSCs are located in the kidney and not BM) have become a well-utilized model system of hematopoiesis. Zebrafish are amenable to medium- and high-throughput analyses and have been used to identify many genes and soluble factors that regulate hematopoiesis.

Single-cell transplantation studies can identify clonal mouse and human hematopoietic cells with the ability to mediate long-term, multilineage engraftment. Several recent research approaches, however, have demonstrated that HSCs are a heterogeneous cell population. Earlier studies used phenotypic cell surface antigens to distinguish HSCs with long-term engraftment ability (LT-HSCs) and those that mediate just short-term engraftment (ST-HSCs). More recent studies (primarily in mice), however, have demonstrated that some HSCs have more myeloid engraftment ability, and some are more lymphoid biased. These subpopulations are maintained through serial transplantation in the mouse.

Pluripotent stem cells and hematopoiesis

Mouse embryonic stem cells (mESCs) were first isolated in 1981. mESCs have been proven invaluable for studies of basic mammalian developmental biology, including hematopoietic development. Unlike adult stem cells (such as HSCs), ESCs are able to undergo self-renewal indefinitely in culture, yet maintain the ability to form all somatic cell lineages (including hematopoietic cells). Studies with mESCs have been invaluable to identify genes that regulate hematopoietic development through gene deletion and/or manipulation. Additionally, diverse hematopoietic cell populations can be derived from mESCs *in vitro* and allow for interrogation of specific genetic and cell signaling pathways that regulate development of specific hematopoietic cell lineages. Notably, attempts to derive HSCs capable of long-term multilineage engraftment largely have failed using mESCs that have not been manipulated genetically. However, overexpression of certain TFs, most notably HoxB4, has been shown to produce hematopoietic cells capable of engraftment in syngeneic recipients. Other TFs have had similar effects.

hESCs were first described in 1998. Like mESCs, hESCs can be maintained indefinitely as a self-renewing population in culture, yet maintain the ability to form all somatic

cell populations. hESCs also have been used to investigate human hematopoiesis. Indeed, key areas of human hematopoiesis are distinct from the murine system. For example, human globin genes undergo 2 switching events during embryonic-fetal development, whereas the mouse undergoes only 1 switching event. hESCs also have raised considerable interest because of the potential for using these cells to produce large amounts of human cells and tissues suitable for research purposes, stem cell transplantation, or transfusion medicine. For example, there has been considerable interest in using hESCs to produce red blood cells (RBCs) or platelets as an adjunct to the living donor blood supply. Additionally, the potential to produce HSCs from hESCs is of great interest. To date, however, although most mature blood cell populations have been produced from hESCs, it has not been possible to demonstrate long-term engraftment of HSCs by transplantation into immunodeficient mice. Genetic manipulation and overexpression of TFs (such as HoxB4) effective in the murine system has not been similarly effective in the human system. Considerable efforts to identify strategies to improve development of HSCs from hESCs are ongoing.

iPSCs are another important cell population. Briefly, iPSCs can be derived from various somatic cell populations, typically by expression of a limited number of “reprogramming genes,” such as *OCT4*, *SOX2*, *KLF4*, and *c-MYC* in human cells, capable of converting somatic cells into cells that look and function like embryonic stem cells. These studies were first done in mouse cells in 2006 and subsequently in human cells in 2007. Like their ESC counterparts, iPSCs have been used to derive diverse hematopoietic cell lineages. Again, to date, HSCs with long-term engraftment potential have not been derived from iPSCs. This field will continue to mature, and there is considerable interest in deriving iPSCs from individuals with different genetic deficiencies to use this system as a human model of genetic disease. Using iPSCs, gene correction strategies or other means to overcome the genetic defect can be evaluated. This may lead to effective therapies based on using iPSCs as a screening resource and would not require direct transplantation of iPSC-derived cells. The generation of disease stage-specific iPSCs in myeloid malignancies has provided insights into the cellular events demarcating the initiation and progression of transformation and a new platform for testing genetic and pharmacological interventions. Future developments may allow for derivation of iPSCs from individuals with hematologic or other diseases and use of these cells to produce autologous replacement cell populations. However, some key challenges that prevent broad translational impact of iPSCs as therapeutic sources in the human hematopoietic system

remain, including unknown short-term and long-term functional capacity of HSCs derived from iPSCs, scalability of small molecule reprogramming methods to generate sufficient cells for therapeutic purposes, and high burden of safety and regulatory requirements and costs for clinical research studies and eventual clinical application involving iPSCs. Continued progress is expected in iPSC research, which may address some of these remaining barriers.

The hematopoietic niche

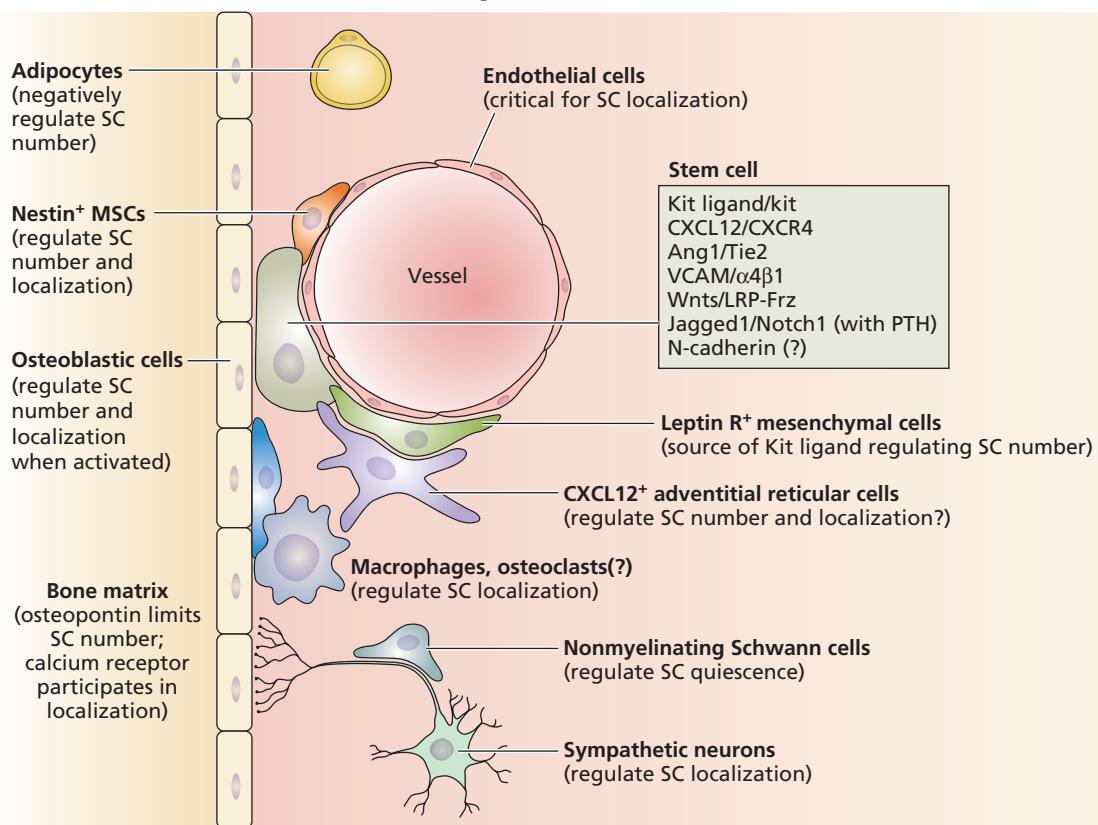
Hematopoietic cell development from HSPCs is regulated by signals provided by the BM microenvironment. The specific constituents of the microenvironment that influence blood cell development are being elucidated, but they can be categorized broadly as heterologous cells, such as mesenchymal cells; endothelial and neural cells; hematopoietic cells; and extracellular matrix. Mesenchymal cells include adipocytes, osteoblasts, leptin receptor-expressing (LepR^+) and nestin-positive cells. Some are part of the continuum of cells that produce bone, and some are perivascular without a clear role

in skeletal biology. Both bone-related and perivascular mesenchymal cells have been shown to influence hematopoiesis.

The posttransplant BM niche is directly relevant to HSPC recovery following transplantation. In vivo imaging studies of the posttransplant niche demonstrated that some transplanted HSPCs are found close to the endosteal surface and osteolineage cells. HSPC-osteolineage cell colocalization in the posttransplant BM niche may be indicative of a regulatory relationship and single-cell RNA-seq shows that HSPC-proximal osteolineage cells have a distinct RNA-seq profile and can regulate HSPC quiescence. Nestin-positive mesenchymal stem cells are important for HSC persistence, and adipocytes variably regulate HSC numbers (Figure 14-5). Previously implicated as negative regulators of HSC number, recent work suggests adipocytes in long bones promote hematopoietic recovery after irradiation by being an important source of stem cell factor (SCF) (Figure 14-5).

The heterogeneous mesenchymal stromal cell (MSC) population plays a significant role in the hematopoietic

Figure 14-5 Bone marrow niche. HSCs localize to perivascular spaces, some of which are near the endosteal surface. A number of mouse models have been used to define specific cell types and gene products that, when manipulated, result in a change in HSC location or number. The cell types are indicated in the figure with the HSC function that their activity appears to modulate. The molecules involved are collected in the gray box, but which cells express these molecules is still being investigated. Some of the molecules are well defined (eg, kit ligand and CXCL12) while others are less well defined and some (N-cadherin) quite controversial.



niche. In human studies, cord blood cocultured with MSCs underwent a median 30-fold expansion of CD34⁺ cells and resulted in significantly improved engraftment. In mice, LepR⁺ cells represent the majority of MSCs. LepR⁺ cells appear to be the main source of new osteoblasts and adipocytes in adult BM and form bony ossicles supportive of hematopoiesis in vivo. LepR⁺ MSCs are the major source of the cytokines SCF that promotes proliferation of cells expressing the SCF-receptor c-kit, and chemokine (CXC motif) ligand 12 (CXCL12), which mediates adhesion of the HSCs in the BM niche. Conditional deletion of the *SCF* gene in LepR⁺ cells leads to depletion of quiescent HSCs and conditional deletion of the gene encoding CXCL12 (*CXCL12*, also called *SDF1*) in LepR⁺ cells leads to HSC mobilization.

Other cell types, such as neural cells of the sympathetic nervous system and nonmyelinated Schwann cells, also play a role in HSC support or localization. The sympathetic nervous system mediates circadian modulation in the number of HSCs moving from BM to bloodstream on a daily basis. Mature hematopoietic cells are also thought to influence HSC function in the BM. Specifically, macrophages help regulate HSC mobilization into blood and T cells are thought to influence HSC engraftment and provide relative protection from immune attack. Megakaryocytes have been shown to be important for maintaining HSC quiescence. Secreted factors, including CXCL4, TGFb1, and thrombopoietin, have also been implicated in this role. Therefore, a complex admixture of cells participates in what is designated as the stem cell niche.

The niche serves several functions important for hematopoiesis. The first is the regulation of stem cell self-renewal, a process that requires expression of molecules, such as SCF and members of the WNT family. The second is control of the number of stem cells, a parameter that is regulated in part by specific extracellular matrix proteins, such as osteopontin, a negative regulator of HSC number. The third is the coordinated regulation of proliferation and differentiation of HSCs; a process that some mouse models have indicated can go awry by changes in the niche and cause myeloproliferative or myelodysplastic phenotypes. The fourth is cell localization, a process that is important in the context of harvesting stem cells by mobilization into the blood or delivery of transplanted HSCs to enable engraftment.

Thus, the HSC niche is a critical aspect of the regulated production of blood cells throughout life. It is a complex tissue in which multiple cell types and extracellular matrix proteins contribute to balance the molecular cues that govern HSC number, self-renewal, and differentiation. By unraveling how stem cells enter and leave the niche, methods to mobilize stem cells for clinical harvest have

been defined (discussed later in this chapter). Ongoing efforts to improve stem cell function and engraftment in the niche and to discern how the niche contributes to disease are contexts in which manipulation of the niche may provide therapeutic potential.

Regulation of hematopoietic differentiation

A complex network of TF and growth factor signaling pathways regulates HSPC self-renewal, lineage commitment, and differentiation. Among TFs, those that are expressed exclusively in blood cells or have restricted tissue-specific patterns of expression play important roles in regulating blood production. Furthermore, acquired driver mutations of these TFs are pathogenic in hematological malignancies such as lymphoma and leukemia. The importance of these TFs is also underscored by the conserved role they play in hematopoiesis through evolution. Over the last 2 decades, this attribute has allowed the function of these TFs to be extensively investigated in animal models. In these models, genes encoding critical TFs have been deleted, modified, overexpressed, and misexpressed. The point of action of some of these TFs is shown in Figure 14-6. A thorough description of the function of these TFs is beyond the scope of this chapter. Some of the key points that arise from these studies are:

1. TFs are divided into families that have similar protein domains.
2. TFs often have protein domains that bind DNA and protein domains that interact with other proteins (other TFs or proteins that control transcription).
3. TFs work in combination with other TFs to activate and/or repress the expression of a large number of genes.
4. TFs are required at discrete stages of hematopoiesis. Any 1 TF can function at multiple stages within a single lineage and can function in more than 1 lineage.
5. Ultimately, TFs work in complicated networks that can be modeled much like semiconductor/computing networks. TFs work in negative feedback loops, feed-forward loops, and cross-antagonistic loops, to mention just 3 such types of interactions.
6. TFs regulate the cell's potential to make blood cells of different lineages, and its potential to proliferate, undergo apoptosis, and self-renew.

More specifically, the TFs SCL/TAL1, LMO2 are required to specify HSCs from mesoderm. The TFs RUNX1 (AML1), TEL1, MLL, GATA2 are required to maintain stem cells once they have been specified. In myelopoiesis, the TFs Pu.1, the C/EBP family (C/EBPa and C/EBPe) GFI-1, EGR-1, and NAB2 all promote the granulocyte-macrophage lineage programs. GATA2 is required in

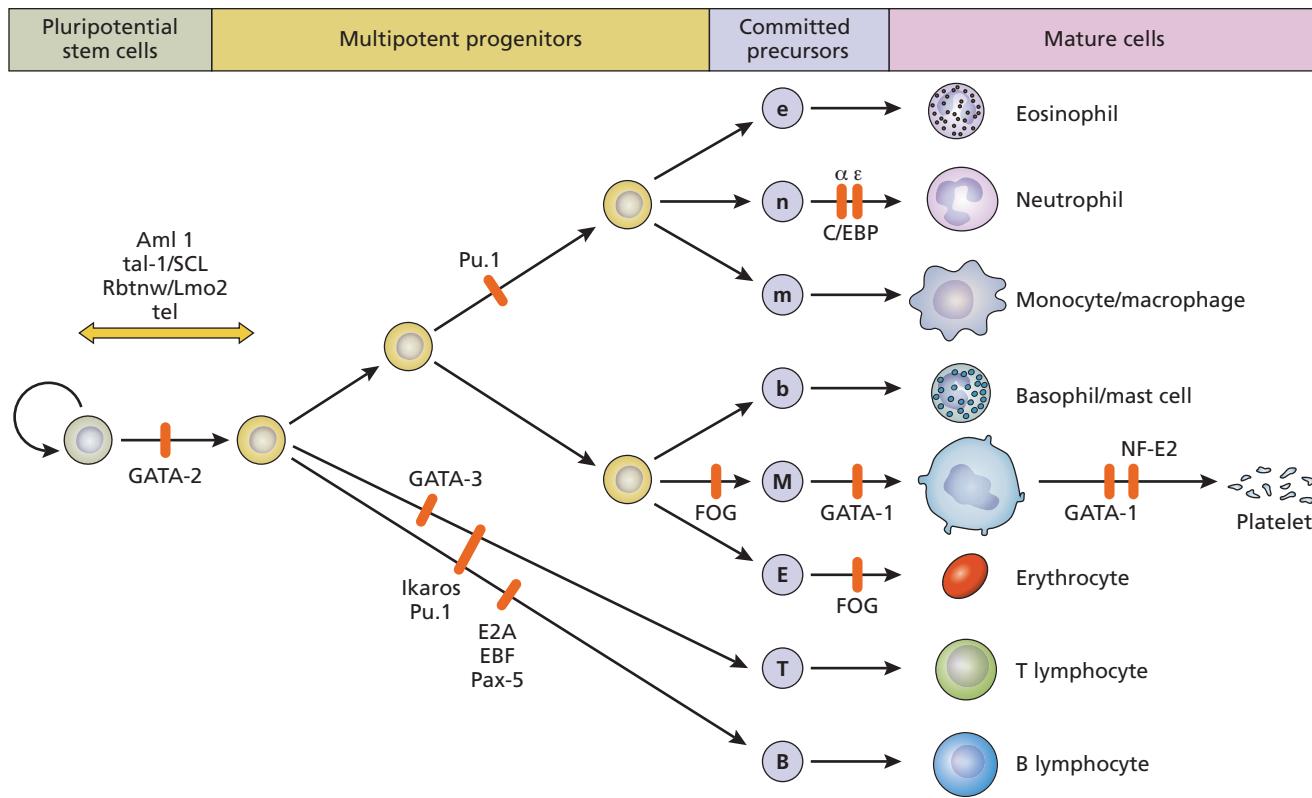


Figure 14-6 A schematic representation of hematopoiesis and where key hematopoietic-specific transcription factors have nonredundant functions, as revealed by gene deletion studies in mice. Thus, for example, the transcription factors GATA2, AML1/RUNX1, TAL-1/SCL, LMO2/RBTN2 and TEL are all critically required in HSCs and loss of function of these genes causes a block (as indicated by the red bar) in hematopoietic differentiation at the HSC level. Similarly, deletions of the other transcription factors cause blocks later in hematopoiesis (as indicated by the red bars).

stem/early progenitor cells but is also required for mast cell differentiation and in the early phases of megakaryocyte-erythroid lineage maturation. Working with GATA2 to promote erythropoiesis and megakaryopoiesis are GATA1, FOG1, SCL, EKLF, p45NF-E2, and Flt-1. In early lymphopoiesis, the TF Ikaros is required. In B lymphopoiesis, the TFs E2A (and its family members), EBF, and PAX5 are required; and finally, the TF BLIMP1 is necessary for plasma cell formation. In T-cell maturation, Notch signaling activates the TF CSL, which works with the TFs GATA3, T-BET, NFATc, and FOXP3. Of note, the TF SCL/TAL1, MLL, RUNX1, LMO2, PU.1, C/EBPα, PAX5, E2A, and GATA1 are all implicated in the pathogenesis of human leukemia.

In addition to TFs, proteins that modulate the epigenetic profile of cells (eg, regulate DNA methylation and histone modifications) and regulate splicing of RNA are also commonly mutated either through loss-of-function or gain-of-function mutations. This also suggests that these proteins play critical roles in normal HSPC differentiation. Examples of proteins that act as epigenetic modulators are provided in Figure 14-7.

Summary

Hematopoiesis involves a tightly regulated set of developmental stages from HSCs to hematopoietic progenitor cells to mature blood cells, which provide all the key functions of the hematopoietic system. Hematopoietic reconstitution during BMT is mediated by a succession of cells at various stages of development. Immediately following transplantation, more mature cells contribute to repopulation. With time, cells at progressively earlier stages of development contribute; with the final, long-term repopulation provided by long-lived multipotent HSCs. Research in induced pluripotent stem cells and in the BM niche offers the potential for a greater understanding of disease biology and for novel therapies to emerge. Clonal hematopoiesis is an age- and chemotherapy-associated disease syndrome associated with clonal expansion of mutant hematopoietic cells and adverse health outcomes. The pathologic mechanisms of how mutations in epigenetic regulatory genes and in other genes disrupt normal hematopoiesis, immune system, and inflammatory pathways in humans remain to be determined.

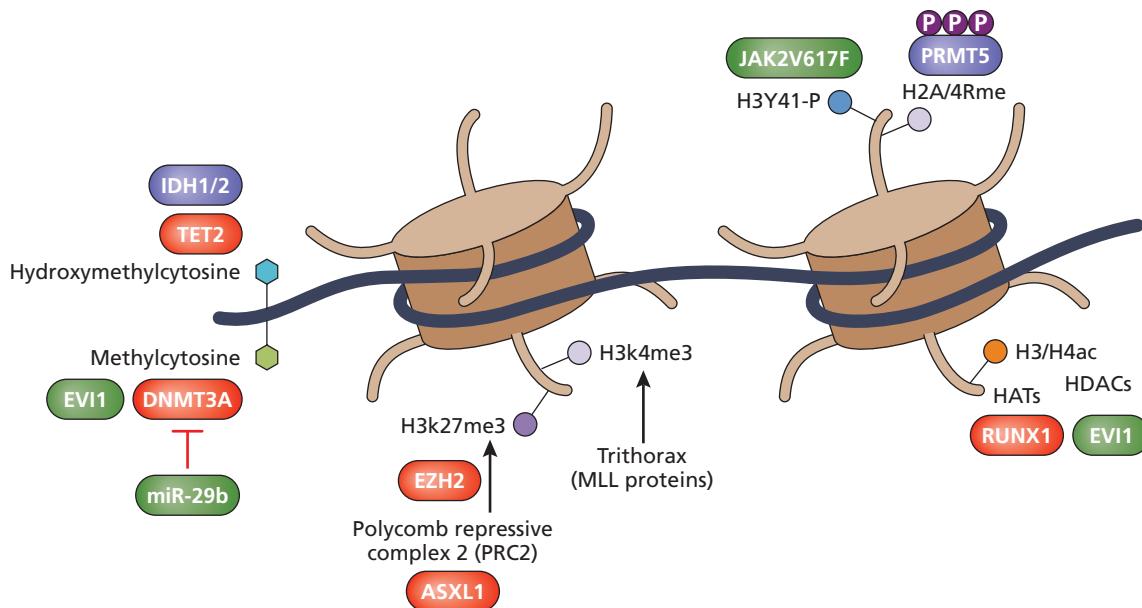


Figure 14-7 Epigenetic modifiers that are altered in sequence or expression in hematopoietic malignancies. Genes outlined in red represent loss-of-function mutants, whereas those outlined in green represent gain-of-function or overexpressed genes. The genes outlined in blue represent mutants that have acquired novel (neomorphic) function. These epigenetic modifiers may impact on DNA methylation (DNMT3A, TET2, IDH1, IDH2), histone (H3 or H4) methylation, or histone acetylation, thereby modifying gene expression across a wide range of targets. HAT, histone acetyltransferase; HDAC, histone deacetylase; me, methyl; ac, acetyl.

KEY POINTS

Development

- Hematopoiesis develops in distinct waves during development.
- Definitive HSCs first develop within the embryo in specialized regions of the dorsal aorta and umbilical arteries and then seed the fetal liver and BM.
- HSC characteristics differ based on their site of development and the age of the organism.

Key features of HSCs

- Ability, at the single-cell level, to reconstitute and maintain a functional hematopoietic system over extended periods of time *in vivo*.
- Self-renewal capacity for life of organism or after transplantation.
- Multipotency: the ability to make multiple types of blood cells.
- Relative quiescence: the ability to serve as a deep reserve of cells to replenish short-lived, rapidly proliferating progenitors.
- *In vivo* transplantation models are currently the only reliable assays of HSC activity and function.
- Acquired somatic mutations in hematopoietic stem cells can lead to clonal expansion (clonal hematopoiesis), a

phenomenon associated with adverse health outcomes including cardiovascular disease and hematologic malignancies.

Key features of hematopoietic progenitor cells

- Inability to maintain long-term hematopoiesis *in vivo* due to limited or absent self-renewal.
- More rapid proliferation and cytokine responsiveness, enabling increased blood cell production under conditions of stress.
- Display lineage commitment, and thereby, limited cell-type production.

Key features of the HSC niche

- Anatomically and functionally defined regulatory environment for HSCs.
- Modulates self-renewal, differentiation, and proliferative activity of HSCs, thereby regulating stem cell number.
- Niche function is important in maintaining HSC integrity; therefore, niche dysfunction may contribute to hematopoietic disease.
- Niches for HSCs are dynamic, changing during development and with physiologic stress.
- HSCs naturally traffic into and out of the niche, a feature that can be exploited for stem cell transplantation or harvesting, respectively.

Clinical transplantation of hematopoietic stem and progenitor cells

Sources of HSPCs in clinical transplantation

Hematopoietic stem cell transplantation (HSCT) provides a fascinating intersection of concepts, including the dose-response relationship of chemoradiotherapy and cancer eradication, stem cell therapy, cancer immunotherapy, and personalized cancer medicine. This section focuses on use of different donor HSPC sources and key differences in the donor products, and it provides a glimpse of where future advances may come from. For more detailed information on the clinical results of transplantation, see Chapter 15.

Autologous transplant

The concept of high-dose therapy plus autologous stem cell transplantation (ASCT) was developed in the 1980s. It was observed that two-thirds of resistant myeloma patients evidenced remarkable antitumor activity after a single dose of melphalan 3 to 4 times higher than the standard dose. Severe and prolonged BM depression caused the death of about one-third of treated patients, a complication usually prevented by autologous BM infusion. The objective of ASCT was to support high-dose therapy in order to reduce the duration and toxicity of severe myelosuppression. Autologous HSCT is most effective when there is direct correlation between chemotherapy dose and tumor response and when the dose-limiting treatment toxicity is myelosuppression. The number of autologous hematopoietic cell transplants (HCTs) has increased steadily since 2000, mainly for the treatment of plasma cell and lymphoproliferative disorders. In acute myeloid leukemia (AML), the relapse advantage of an autologous transplant was offset by prolonged marrow aplasia and an excess of nonrelapse mortality, which has precluded general acceptance of ASCT as postremission treatment in AML. However, recent retrospective studies suggest that among AML patients with intermediate cytogenetics who were in their first complete remission, the leukemia-free survival rate of autologous HSCT did not differ significantly from that of human leukocyte antigen (HLA)-matched unrelated-donor HSCT.

Alloreactivity as a therapeutic principle in the treatment of hematologic malignancies

Allogeneic HSCT represents a potentially curative treatment modality in a range of hematologic malignancies. See Chapter 15 for disease-specific applications of allogeneic transplantation. In allogeneic HSCT, the conditioning regimen eradicates malignant cells, ineffective hematopoi-

etic cells in the case of nonmalignant disorders, and host immune cells that may reject the donor cells. Although HSCT was originally regarded as a way of rescuing patients from therapy-induced marrow aplasia, it is now accepted that alloreactive donor cells produce a substantial graft versus tumor (GVT) effect that contributes to cancer eradication.

Alloreactivity denotes the immunologic reactions that occur when tissues are transplanted between 2 individuals within the same species. Allogeneic hematopoietic cell transplantation evolved as a means to harness the immune system to treat hematologic malignancies in patients who fail to respond to standard chemotherapy. The biological foundation of this immunotherapy is the graft-versus-leukemia effect, which is primarily mediated by donor T cells present in the graft. The combination of tumor burden reduction, immunosuppression and provision of a diverse repertoire of alloreactive T cells can produce remarkable clinical responses, but this response comes at the price of graft-versus-host disease (GVHD), whereby healthy host tissues are attacked. For a detailed description of the clinical spectrum of GVHD see Chapter 15. Clinical studies have shown that patients who develop GVHD have a lower risk of relapse of the malignant disease and that additional donor lymphocyte infusions can induce durable remissions in patients with relapsed disease after the transplant. These observations indicate that GVHD lies on the same immunologic continuum as a GVT effect and balancing the 2 phenomena is a major clinical conundrum in the transplant field.

Demographic shifts and drifts

The clinical utilization of transplantation is dynamic, and transplant practices are influenced by development in prognostication strategies and the development of novel therapies. The drop in allogeneic HSCT for chronic lymphocytic leukemia in the past 2 years in EBMT and BMT-CTN registries is remarkable and reminds us of the drop seen in chronic myelogenous leukemia transplants once kinase inhibitors became available. Currently, the majority of allogeneic HSCTs registered in the Center for International Blood and Marrow Research (CIBMTR) are for the indication of AML and increasing numbers of patients are being transplanted in first remission in AML due to better prognostication strategies that predict disease behavior and relapse potential. Furthermore, individuals with comorbidities and those 70 years of age and older are now eligible to undergo allogeneic HSCT, following the introduction of reduced-intensity or nonmyeloablative conditioning regimens, which have resulted in a decrease in regimen-related morbidity and mortality. For a detailed explanation of conditioning regimens, see Chapter 15. Between 1991 and

1997, 7% of allogeneic HCTs were performed in patients over 50 years of age; between 2000 and 2015, this percentage increased to 38%. In 2015, 25% of all allogeneic HCT recipients were patients over 60 years old, compared to 5% in 2000. Perhaps most impressive is a growth in allogeneic HSCT using haploidentical donors, an increase of over 200% in the last 5 years. A haploidentical donor shares exactly 1 HLA haplotype with the recipient and is mismatched for a variable number of HLA genes, ranging from 0 to 5, on the unshared haplotype.

Nonmalignant applications of HSCT

HSCT is also an established treatment for congenital or acquired BM failure, immunodeficiency states, and autoimmunity. In these cases, the GVT effect is not desired, and prevention of GVHD is a priority. HSCs can also act as “therapeutic vehicles” to replace defective or missing enzymes, such as adenosine deaminase in SCID, or to overcome monogenic disorders such as hemophilia or sickle cell disease (SCD) by gene transfer. Alternatively, allogeneic stem cell transplant presents a curative option for severe β -hemoglobinopathies. In β -thalassemia, myeloablative conditioning (MAC) with HLA-matched sibling donor cells is the treatment of choice, providing an excellent outcome and utilized in many pediatric patients with a compatible intrafamilial donor. The toxicity of MAC was impeding the progress of transplantation in SCD but use of nonmyeloablative conditioning with matched sibling donors is resulting in excellent survival and long-term quality of life in SCD. These patients show coexistence of host and donor cells referred to as persistent mixed chimerism. This phenomenon of tolerance seen in mixed chimeric states without development of GVHD is being leveraged in the solid organ transplant world. Several clinical studies have combined hematopoietic cells in conjunction with solid organ transplants, as microchimerism facilitates the establishment of transplanted organ tolerance and allows discontinuation of immunosuppression that these patients are usually committed to for life.

The European Society for Blood and Marrow Transplantation (EBMT) registry confirms that activity in HSCT for severe autoimmune diseases is increasing in spite of adoption of biologic therapies, with the major indication being multiple sclerosis followed by systemic sclerosis. The combination of lymphotoxic chemotherapy, such as cyclophosphamide and antithymocyte globulin, leads to persistently reduced levels of putative pathogenic autoantibodies and autologous HSCT can reestablish immunological tolerance by an increased number of regulatory, FoxP3-positive T cells, which are important in the preservation of tolerance. Further prospective studies are required to

compare HSCT with evolving modern treatments. Studies are also required to refine conditioning regimens and late effects of HSCT need to be considered.

Bone marrow versus mobilized peripheral blood

Since the 1990s, peripheral blood stem cells have steadily surpassed BM as a stem cell source due to faster engraftment and practicability. The Center for International Blood and Marrow Transplant Research reported that, in the period from 2007 to 2011 about 70% to 80% of adult allogeneic transplant recipients received peripheral blood stem cells.

A systematic review, which included 9 randomized controlled trials and 1,521 related and unrelated donor allogeneic BMT recipients with hematologic malignancies, demonstrated that overall survival and disease-free survival between the 2 graft sources were comparable. However the role of BM as a preferred source has been raised, based on a Blood and Marrow Transplant Clinical Trial Networks (BMT CTN 0201) randomized trial demonstrating that patients undergoing matched unrelated donor BMT with MAC and standard GVHD prophylaxis (methotrexate and calcineurin inhibitors) with a peripheral blood stem cell graft experienced more chronic GHVD than those who received BM (53% vs 41%, $P=0.01$). There was no difference in relapse, disease-free survival, or overall survival between the 2 treatment arms; although BM recipients had a higher incidence of graft failure (9% vs 3%, $P=0.02$). In addition, BM recipients reported better psychological well-being, less burdensome chronic GHVD symptoms and were more likely to return to work at 5 years after BMT. However, donor preference (30% of screened donors declined randomization in the CTN trial), as well as an increasing number of therapeutic modalities for GVHD, makes the adoption of BM over peripheral blood a difficult practice to implement in unrelated transplants.

How do we match donor and recipient?

When PBSCs are selected for allogeneic transplantation, HSPCs have to be matched to avoid the alloimmune response of donor immune cells against host (GVHD) and, conversely, the alloimmune reaction of the host against donor cells leading to graft rejection. The major genetically encoded loci mediating alloimmune responses are the cell surface HLAs encoded on chromosome 6. These encode major histocompatibility complex (MHC) class I (HLA-A, -B, and -C) and class II (DR, DQ and DP, DM and DO) antigens. MHC class I antigens are present on all cells; class II antigens are only present on immune antigen-presenting cells. Aside from the HLA genes, there are a

large number of other genes encoding cell surface proteins that collectively are termed *minor histocompatibility antigens*. As individual proteins, they play a more modest role in an alloimmune response but collectively they are likely to direct both GVHD and graft-versus-disease responses that are not completely understood.

To identify potential HSPC donors and inform donor choice, high-resolution molecular typing has replaced serotyping. Detailed national guidelines exist to guide donor choice based on molecular typing (United States: <https://bethematchclinical.org/transplant-therapy-and-donor-matching/hla-typing-and-matching/>). A complete HLA-matched sibling donor is almost always the first choice of allogeneic donor. HLA-matched sibling donor cells cause less GVHD and therefore less morbidity and mortality. Furthermore, matched sibling donors often are easier logically to coordinate for timing of transplantation. Using Mendelian laws of inheritance, the likelihood that a sibling pair is HLA identical would be exactly 25%. Crossover phenomena during meiosis explain unusual cases of aberrant recombination of HLA antigens resulting in a probability slightly lower than 25%.

Multiple studies examining the impact of donor age on transplant success show that younger donors result in better outcomes for patients, resulting in a donor age limit of 60 years in the National Marrow Donor Program registry. Recent elucidation of the presence of clonal hematopoiesis of indeterminate potential (CHIP) in asymptomatic elderly subjects has sparked great interest in the role of CHIP in increasing risk of myeloid malignancies. A recent study points to an association between CHIP and the development of myeloid malignancies. Of the 401 patients who received autologous stem cell transplants for their lymphoma treatment, patients with CHIP had significantly lower 10-year overall survival than patients without CHIP.

Given diminishing family sizes and the increasing age of patients, medically fit sibling donors are often not available. Thus, many allogeneic transplants rely on matched unrelated donors. Such donors are matched at HLA-A, -B, -C, DRB1 and DQB1 loci. Ten out of 10 matches are recommended; where this is not possible, a single mismatch at HLA-A, -B, -C, DRB1 and DRQ1 is acceptable. Fortunately, there are 2 features in the HLA system that may make finding a match easier, as they allow us to “predict” whether a donor will be matched or mismatched from the available information. In *linkage disequilibrium*, alleles occur together with a greater frequency than would be expected by chance. Linkage disequilibrium is more frequently observed between loci that are in close proximity (eg, be-

tween HLA-B and -C and HLA-DRB1 and -DQB1). A *haplotype* is a group of genes inherited together. There are a number of common haplotypes in different ethnic groups.

Despite matching for HLAs, unrelated donors are much more likely to be a mismatch at minor histocompatibility antigens. However with appropriate GVHD prophylaxis, multiple prospective trials have now demonstrated similar survival outcomes between matched unrelated donor and matched related donor transplants in AML.

Donor-specific antibodies

Allogeneic hematopoietic stem cell recipients may have preformed antibodies directed against foreign HLA antigens. The use of partially HLA-mismatched allogeneic hematopoietic stem cell donors allows for the possibility of the presence of circulating HLA donor-specific antibodies (DSAs) in the recipient. Anti-HLA Abs against mismatched HLA antigens have an important role in the development of graft failure. Common exposures resulting in development of DSAs include pregnancy, blood product transfusion, and previous organ or blood transplantation. DSAs tend to be of higher intensity when directed against haploidentical first-degree relatives. DSA assessment requires frequent monitoring because their relative strength can change over time. Although the criteria that constitute a prohibitive DSA are unknown, desensitization techniques can result in engraftment.

Killer immunoglobulin-like receptor (KIR) ligand

NK cells constitute a critical component of innate immunity, being the first in the line of defense against tumors and viral infections; are able to suppress or amplify T-cell alloreactivity; and are among the earliest lymphocyte subsets to reconstitute and achieve functional maturity (within weeks) after HCT. Killer immunoglobulin-like receptors (KIRs) control NK function and are encoded by the highly polymorphic, multimembered KIR gene family. Interaction between self-specific inhibitory KIR and cognate HLA ligands is fundamental to NK education.

In patients with AML who undergo HCT, lack of HLA ligand for donor KIR is associated with superior NK reactivity and lower relapse as a result of lack of NK inhibition. A recent study of 1,328 patients with AML who received HLA-compatible allografts, donor-recipient *KIR3DL1/HLA-B* subtype combinations with weak or no inhibition in vitro were associated with significantly lower relapse and higher survival than strong inhibition combinations. KIR and HLA titrate NK inhibition in a predictable, subtype-specific manner, which translates to

hierarchical leukemia control. Therefore, refining donor selection algorithms to include *KIR3DL1/HLA-B* subtype analysis to avoid strong inhibition donors may reduce relapse and improve survival.

How do we select mobilization strategies?

Following the observation that chemotherapy administration resulted in a transient surge in circulation of stem cells during hematopoietic recovery, early stem cell mobilization techniques relied on chemotherapy alone. The discovery and manufacture of hematopoietic cytokines transformed stem cell collection. G-CSF, the most potent of the myeloid growth factors, works by inducing the release of various proteases into the marrow, which then cleave adhesion molecules such as SDF-1, releasing hematopoietic stem cells into the blood. The use of chemotherapy before G-CSF generally produces higher stem cell yields, and in theory may reduce tumor contamination of the stem cell product but is not an integral component of mobilization. It is utilized in treatment plans for lymphoma within the initial 3 to 6 cycles of chemotherapy with very low relapse rates of <3%.

The novel stem cell–mobilizing agent plerixafor has recently provided another mobilization option for the transplantation community. In 2008, plerixafor was approved for use in the United States in combination with G-CSF for the mobilization of hematopoietic stem cells in patients with non-Hodgkin lymphoma and multiple myeloma undergoing high-dose chemotherapy followed by autologous stem cell rescue. Plerixafor is a reversible CXCR4 antagonist that allows the release of stem cells from the marrow by disrupting the interaction of CXCR4 with SDF-1. Administration of plerixafor in conjunction with G-CSF augments mobilization of CD34⁺ cells into the peripheral blood (PB), with a peak effect 4 to 9 hours after administration but a much longer sustained effect, allowing for later initiation of apheresis.

The stem cell population mobilized by the combination of plerixafor and G-CSF differs from that mobilized by G-CSF alone. Plerixafor-mobilized stem cells have a higher proportion of cells in the growth phase, primitive CD34⁺CD38⁻ progenitor cells, B and T lymphocytes, dendritic cells, and NK cells. These characteristics suggest that plerixafor-mobilized cell products may have greater capacity to repopulate the marrow and reconstitute the immune system compared with grafts mobilized by G-CSF alone. Alternative drugs that modulate the SDF-1/CXCR4 axis have shown promising results in early human studies.

Stem cell factor binds to c-kit on HSC and activates multiple downstream pathways, including adhesion. Re-

combinant human SCF used in combination with G-CSF has been shown to increase stem cell yield in poor mobilizers and is approved in Canada and New Zealand. Rare severe reactions related to mast cell activation have limited its use. Adhesion molecules such as very late antigen-4 (VLA-4) receptors on HSC mediate interaction with the BM vascular endothelial cells, maintaining HSC within the marrow microenvironment. VLA-4 antagonists have shown efficacy as mobilizing agents in animal studies; and natalizumab, a recombinant humanized monoclonal antibody against the α_4 subunit of VLA-4, approved for the treatment of multiple sclerosis and Crohn disease, increased peripheral blood CD34⁺ cells in patients. A recent study demonstrated a positive correlation between parathyroid hormone levels and the number of circulating HSCs. Stimulation with parathyroid hormone showed HSC mobilization comparable with that produced by G-CSF in animal models and was effective and well-tolerated in a phase 1 study. Sphingosine-1-phosphate (S1P) is a bioactive phospholipid stored and released into blood mainly by erythrocytes. S1P in the plasma creates a gradient that facilitates the egress of BM HSCs. It has been shown that an elevated plasma S1P level resulting from hemolysis acts as a critical chemoattractant to the BM HSCs. The S1P(1) agonist SEW2871 enhanced plerixafor-induced HSC mobilization in animal models. HSC mobilization promotes hypoxia within the BM microenvironment, which leads to stabilization of HIF-1 α . HIF-1 α induces vasodilation in the BM sinusoids and enhancement in HSC mobilization. A recent study found that stabilization of HIF-1 α with FG-4497-a propyl hydroxylase inhibitor, when combined with G-CSF and plerixafor, led to a 6-fold increase in mobilization of HSCs in mice.

Optimal cell dose

The correlation between the number of stem cells infused for aHSCT and engraftment kinetics is well established. Administration of CD34⁺ cell doses of $<1.5 \times 10^6$ to 2.5×10^6 /kg leads to delayed neutrophil and platelet recovery and administration of doses of $<1 \times 10^6$ /kg has been associated with increased RBC transfusion requirements and even permanent loss of engraftment. Infusion of $>3 \times 10^6$ to 5×10^6 cells/kg is associated with earlier neutrophil and platelet engraftment. A recent post hoc analysis of higher stem cell doses in patients undergoing aHSCT demonstrated that CD34⁺ cell doses of $>6 \times 10^6$ /kg were associated with improved long-term platelet recovery and reduced blood transfusion requirements, although there was no significant difference in time to platelet recovery to 20×10^9 /L. More research is needed to determine

the impact of higher cell doses on engraftment kinetics and to evaluate whether time to collection and stem cell quality, not simply quantity, may play an important role as well.

Predicting poor mobilization

Optimal mobilization requires the collection of the targeted stem cell dose with the minimum number of apheresis sessions required, low cost, and avoidance of mobilization-related complications, such as hospitalization for febrile neutropenia. Mobilization failure rates with traditional strategies are as high as 35%. Risk factors for failure include advanced age, previous radiation therapy or extensive chemotherapy, previous treatment with lenalidomide or a purine analog, previous mobilization failure, and low preapheresis circulating CD34 cell counts. Diabetes mellitus also contributes by alteration of the hematopoietic niche via a sympathetic denervation. A recent multivariate analysis showed that donors with CHIP required significantly more days to collect an adequate number of stem cells and were more likely to fail peripheral mobilization and require BM harvest.

A direct linear correlation was reported between PB CD34⁺ cell count and overall collection, such that a doubling of the preapheresis CD34⁺ count doubles the number of CD34⁺ cells collected during apheresis. Thus, identification of patients with suboptimal preapheresis PB CD34⁺ counts may allow for the salvage of initial mobilization attempts with novel agents, thereby reducing the high failure rates seen with traditional strategies.

Is a matched related donor equivalent to an unrelated donor?

The first successful unrelated donor (UD) transplant was performed in the United States in 1973. Since then, >60,000 UD transplants have been performed, with long-term survivors of >25 years. Petersdorf and Flomenberg demonstrated striking prognostic efficacy of high-resolution typing and, consequently, only donors with 9/10 or 10/10 HLA matches (4 digits per allele) are routinely utilized. As the practice of UD transplantation has become commonplace, numerous studies have now shown that survival following a UD transplant is not different from that using an HLA-identical sibling. Matched related donor BM transplantation does result in faster engraftment and more rapid immune reconstitution, resulting in fewer severe infections. This may in part be due to the short and limited GVHD prophylaxis in this setting. In the setting of UD transplantation, the degree of HLA matching and avoidance of DSAs are the main drivers of donor selection. In addition,

prioritization is usually given to younger donors, as donor age appears to be the only non-HLA factor affecting survival. In the CIBMTR, unrelated donor transplants have increased steadily and surpassed related donor HSCT since 2006.

No patient without a donor: surmounting the HLA barrier

There is significant ethnic variation in the availability of unrelated donors, ranging from about 19% for African Americans to 80% or more for Caucasians of northern European origin. For patients without a matched unrelated donor option, alternative donors such as haploidentical donors and cord blood stem cells may need to be considered. Table 14-1 provides a comparative overview of the donor sources currently available.

Haploidentical transplant

Nearly all patients have an available haploidentical donor because all biologic parents and children of a patient are haploidentical and each sibling or half-sibling has a 50% chance of being haploidentical. Historically, haploidentical transplants have been impeded by an intense bidirectional alloreactivity of T cells leading to a high incidence of both graft failure and GVHD. However, a modern transplant technique pioneered by the Johns Hopkins group has incorporated high-dose posttransplant cyclophosphamide (PTCy) on days 3 and 4, resulting in a reduced incidence of acute GVHD to levels consistent with, and chronic GVHD to levels below that, of HLA-matched transplantation. Early after transplant, alloreactive T cells are susceptible to alkylator therapy-induced death, while hematopoietic stem cells and nonalloreactive T cells are spared because of their quiescence and ability to express aldehyde dehydrogenase, which can metabolize cyclophosphamide to an inactive metabolite. Consequently, nonrelapse morality after haploidentical BMT with PTCy has declined to a level comparable to HLA-matched transplantation. The presence of clinically significant DSA in the recipient, directed against donor HLA, has been reported to induce graft failure in up to 75% of haploidentical and all efforts are usually made to avoid these donors.

The ease of PTCy has led to its adoption across the world, with data from EBMT and CIBMTR both showing a rapidly increasing trend for haploidentical HSCT over the last 5 years, as opposed to more stagnant levels of umbilical cord blood (UCB) transplantation over the same time period. So, it may turn out that PTCy is the great equalizer of adult stem cell sources by abolishing the det-

Table 14-1 Comparison of donor graft sources

	Matched related		Matched unrelated		Umbilical cord	Haploididential
	Peripheral blood	Bone marrow	Peripheral blood	Bone marrow		
Chronic GVHD	+	+	+++	++	+++	+++
Time to engraft	Average (+)		Average (+)		High (+++)	High (++)
Graft failure	Standard risk (+)		Standard risk (+)		Higher risk (++)	Higher risk (++)
Immune reconstitution	Fast	Slow	Fast	Slow	Very Slow	Very Slow
Cell dose	High	Higher	High	Higher	Low	High
HLA mismatch	7/8 or 8/8		7/8 or 8/8		4/6	Haplotype
Graft versus tumor	+	+	++	+	++	++
Adoptive cellular immunotherapy options	Yes		Possible but not always		No	Yes
Rapid availability	Yes		No		Yes	Yes
Cost	Standard		Standard		High	Standard

Data from Petit I et al, *Nat Immunol*. 2002;3(7):687–694; Giralt S et al, *Biol Blood Marow Transplant*. 2014;20(3):295–308; and DiPersio JF et al, *Blood*. 2009;113(23):5720–5726.

rimental effect of HLA or minor histocompatibility mismatches on the outcome of allogeneic SCT.

Cord blood transplant

Discovery of UCB as a third source of HSPCs occurred in the wake of another nuclear accident in the 1980s, the Chernobyl catastrophe. Broxmeyer et al explored the hematopoietic potential of UCB HSPCs for clinical use based on the knowledge that these HSPCs could be maintained in long-term cultures for many weeks, with self-renewal and progenitor cell proliferation potential in vitro. The resulting multi-institutional clinical collaboration led to the first successful UCB transplantation for treatment of Fanconi's anemia in 1988.

UCB HSCTs require less strict HLA matching and are associated with a reduced incidence of chronic GVHD due to an immunologically naïve donor-derived T-cell repertoire. The limitations of cord blood transplants include slower hematopoietic recovery and delayed immune reconstitution because of the limited number of progenitor cells in each unit. When UCB units lack the requisite number of progenitor cells, the use of 2 partially matched UCB units provides acceptable results. Two recent randomized controlled trials in children and young adults have compared transplantation using 2 UCB units versus a single UCB unit and concluded that single-UCB transplantation with adequate cell dose is preferable to 2 UCB units unless a single unit of adequate cell dose is not available. It should be recognized that the definition of "adequate" cell dose is $\geq 2.5 \times 10^7$ nucleated cells/kg recipient weight, with higher doses preferred for greater HLA mismatch.

Cells to prevent or treat relapse after allogeneic stem cell transplantation

Malignant cells can recruit immunosuppressive cells and produce or induce soluble inhibitory factors that create a tumor microenvironment in which cancers are able to avoid immune-mediated killing. This can include dendritic cell dysfunction, defective tumor antigen presentation, checkpoint pathway activation, and resistance of tumor cells to death through altered metabolism. All of these are therapeutic opportunities in efforts to lower the probability of disease relapse. A comprehensive review of adoptive cell therapy is provided in Chapter 15.

Lymphocytes

Unmanipulated, unselected donor lymphocyte infusion is a well-established therapy in the management of post-transplant relapse and forms the benchmark against which many newer cellular therapies have been judged. Cytotoxic T-lymphocyte clones that are directed against target tumor-associated antigens can be expanded and used to treat or prevent malignancy after HSC, best exemplified by Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes in the setting of posttransplant EBV-associated lymphomas. The most clinically advanced cellular therapy is the use of T cells retrovirally engineered to express a chimeric antigen receptor (CAR), which can achieve high response rates and long-term antitumor activity. Remarkable complete remissions have been obtained with autologous T cells expressing CD19 CARs in patients with relapsed, refractory B-cell acute lymphoblastic leukemia, chronic lymphocytic

leukemia and non-Hodgkin lymphoma. However, the use of allogeneic T cells poses unique challenges owing to their potential alloreactivity.

Monocyte-based cellular therapy

Monocyte-derived dendritic cells are potent antigen-presenting cells that educate T cells to recognize tumor antigens, which results in the production of tumor-specific cytotoxic T lymphocytes. Most trials of dendritic cell vaccines to date have been conducted in the autologous setting, although this approach has also been adapted for allogeneic use. Multiple antigen sources can be used in dendritic cell vaccines, including tumor cell lysates, apoptotic bodies, exosomes or fusions, tumor-derived RNA, and tumor-targeted proteins or peptides. Leukemia-associated antigens, such as WT1, have been most frequently used.

NK-based cellular therapy

NK cells are CD56⁺, CD3⁻ innate immune effectors that are capable of responding to and eradicating pathogen-infected and tumor cells rapidly and without recruitment of T cells. The antitumor effect associated with NK cells has been best described in the setting of HSCT, particularly in regard to killer immunoglobulin receptor biology where donor-recipient mismatched alloreactive NK cells can mediate a graft-versus-leukemia effect.

Ultimately, combinations of cellular therapies, or the combination of these therapies with novel agents such as epigenetic modifying agents, checkpoint inhibitors, and/or standard chemotherapy, are likely necessary to achieve the greatest benefits.

Graft manipulation

The concept of dissecting GVHD and GVT is the mother lode in stem cell transplantation. T cells are the major component of the hematopoietic stem cell graft, exerting an adaptive or innate immune response. Graft manipulation is commonly done via depletion of T cells that are implicated in GVHD, or less commonly, expansion of regulatory T cells that would confer host tolerance. Methods of ex vivo T-cell depletion include negative selection of T cells, which can be performed with antibodies, or an alternative strategy is CD34⁺-positive selection using immunomagnetic beads. While aggressive T-cell depletion significantly reduces the risk of acute and chronic GVHD, it comes at the cost of increased risk of relapse, graft failure, and infection, and this has hindered its widespread adoption. More targeted T-cell depletion and strategies to add back specific T-cell populations as well as suicide-gene programming of add-back T cells, are all investigational approaches to mitigate relapse risk and enhance immune reconstitution.

Ex vivo stem cell expansion

Many agents have been tested for HSC expansion, but most have failed to increase the quantities of long-term repopulating HSCs. Cytokines such as FMS-like tyrosine kinase 3, thrombopoietin, and SCF were found to be necessary but not sufficient for HSC self-renewal. *HOXB4* was cloned into murine BM HSPC ex vivo and resulted in HSC expansion and long-term multilineage reconstitution after transplantation. However, a preclinical study in larger primates was complicated by vector-mediated insertional mutagenesis and leukemia in these higher animals.

Notch ligand-mediated expansion and mesenchymal stromal cell coculture caused progenitor cell proliferation with significantly shortened early hematopoietic recovery, but at the expense of long-term repopulating HSC. 16,16-Dimethyl prostaglandin E₂ (dmPGE₂) was previously identified as a critical regulator of HSC homeostasis. Ex vivo modulation with brief exposure of HSC to dmPGE₂ demonstrated success in murine models and shortened neutropenia in UCB transplants in humans. Further clinical studies are exploring HSC expansion agents such as StemRegenin1 and human umbilical vein endothelial cell coculture.

The lack of long-term engraftment with these coculture systems could be attributed to loss of long-term repopulating ability during the ex vivo culture period. An alternate explanation is that without coinfusion of immunocompetent T cells, the expanded graft cannot compete successfully for long-term engraftment. Recent studies of nicotinamide as an ex vivo expander have tried to address this issue. UCB-derived hematopoietic stem and progenitor cells were expanded in the presence of nicotinamide and transplanted with a T-cell-containing fraction containing both short-term and long-term repopulating cells. In a trial of 12 patients, this product provided rapid short-term engraftment and stable long-term multilineage hematopoiesis. A multi-institution phase 3 study is currently accruing.

Summary

Over the last 50 years, more than 1 million hematopoietic stem cell transplants have been performed. In this time, HSCT has evolved from an experimental therapy leading to a Nobel Prize in Medicine awarded in 1990 to E. Donnall Thomas to the standard of care for many malignant and nonmalignant diseases. Improved donor selection, tailored conditioning and better supportive care have reduced transplant-related mortality. Moreover, with reduced intensity conditioning and the burgeoning use of alternative donor sources, transplant access has now expanded to nearly any patient who needs it. The major issues facing

transplantation are to better understand and harness the immune response to provide long-lasting protective immunity against tumor recurrence, which remains the major cause of posttransplant mortality. Use of additional cellular therapies, including CAR-T cells and modulation of costimulatory pathways, will allow more specific targeted immunotherapeutics in the near future.

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15

Clinical hematopoietic cell transplantation and adoptive cell therapy

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Historical perspective

The advent of the atomic era and the potential for large-scale human exposure to ionizing radiation either accidentally or intentionally resulted in a dramatic increase in basic and preclinical research in hematopoiesis and hematopoietic cell transplantation (HCT) as a therapeutic strategy against exposure to lethal radiation. The following seminal observations were required to develop the field:

1. Safety and feasibility of human bone marrow infusion
2. Ability of normal stem cells to reconstitute a lethally radiated host
3. Recognition of a potential graft-versus-tumor (GVT) effect operative in animal models and humans
4. Safety and feasibility of cryopreserved autologous bone marrow in reconstituting lethally radiated hosts

Notwithstanding these early observations, the initial clinical experience with HCT was dismal, with most patients succumbing to transplant-related complications. It was not until the discovery and identification of human leukocyte antigens (HLAs), as well as improvements in supportive care with antibiotics and antifungals, that successful HCT could be a reality for sufficient numbers of patients to warrant large-scale study. A landmark paper from Thomas et al, demonstrating that long-term remission could be achieved in patients with refractory acute leukemia with the use of high-dose chemoradiotherapy followed by infusion of HLA-identical sibling bone marrow, marked the beginning of HCT.

The rationale for high-dose cytotoxic chemotherapy stems from the steep dose-response curve of alkylating agents and radiotherapy and tumor cell response in human tumors. Doubling the dose of alkylating agents increases tumor cell kill by a log or more and increasing the dose of alkylating agents by 5- to 10-fold overcomes the resistance of tumor cells against lower doses. In 1978, investigators from the National Cancer Institute were the first to report the use of high-dose chemotherapy followed by autologous HCT for patients with relapsed lymphoma. These encouraging results were the initial clinical evidence leading to the widespread application of autologous HCT. McElwain and Powles demonstrated a similar dose-response curve for melphalan in patients with myeloma, which led to the beginning of high-dose therapy for myeloma, the most common indication for autologous HCT.

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The hematopoietic stem cell

Hematopoietic progenitor cells (HPCs) are capable of reconstituting and maintaining a complete and functional hematopoietic system over extended periods of time. They are characterized by 3 intrinsic properties: extensive proliferative capacity, pluripotency (the ability to differentiate into all blood cell types), and self-renewal capacity (the ability to replace the cells that became progressively committed to differentiation). The HPC has been functionally defined by the following:

1. Ability to form multilineage colonies in semisolid soft agar medium
2. Ability to form colony-forming units after being maintained in culture for a minimum of 5 weeks
3. Ability to provide long-term (>4 months) repopulation of all blood lineages of myeloablated host mice

HPCs account for 1 in 10,000 bone marrow cells, and during normal steady-state hematopoiesis, are in the G₀ phase of the cell cycle. Through chemical signals, they are recruited into active hematopoiesis and undergo a series of maturational cell divisions that culminate in the generation of progenitor cells that have progressively limited self-renewal, are proliferative, and have the potential to differentiate into different cell types.

Hematopoietic cells develop in vivo in intimate association with a heterogeneous population of stromal cells and an extracellular matrix that constitute the microenvironment of the bone marrow. Fibroblasts, smooth muscle cells, adipocytes, osteogenic cells, and macrophages compose the stromal cell compartment. Extracellular matrix molecules of 7 distinct families have been identified—including collagens, proteoglycans, fibronectin, tenascin, thrombospondin, laminin, and hemonectin. Within the marrow microenvironment, 2 types of niches have been described that favor HPC self-renewal vs differentiation. The osteoblastic niche is located in the periosteal region of the bone cavity; and the vascular niche involves vascular sinusoids within the bone marrow. A complex network of transcription factor and growth factor signaling pathways tightly regulates HPC recruitment, lineage commitment, and differentiation. The advent of flow cytometric techniques has allowed cell surface markers to be used to prospectively isolate cell populations with selective functional properties.

Stem cells migrate from sites of blood cell production, circulate in the blood, home, and enter other supportive sites. Control of these processes currently is not well understood but appears to involve lectins, integrin adhesion molecules, chemokines, and their receptors. The ability to alter these interactions with such agents as granulocyte

colony-stimulating factor (G-CSF) or CXCR4 antagonists allows for “mobilization” of hematopoietic stem cells (HSCs) into the peripheral blood system and their collection by apheresis for transplantation.

The hematopoietic cell transplantation process

The HCT process begins with the administration of a conditioning regimen of chemotherapy and sometimes radiation to eradicate a malignant disorder or a poorly functioning bone marrow. Allogeneic HCT also requires the administration of immune-suppressive, lymphotoxic chemotherapy to promote donor engraftment, sometimes with T cell-depleting antibody therapy to reduce the risk of graft-versus-host disease (GVHD). The conditioning regimen is followed by reinfusion of HSCs from the patient (autologous) or a related or unrelated donor (allogeneic, syngeneic if identical twins). Intense medical support is required as patients recover from the effects of the conditioning regimen and during the period of immune suppression that occurs while the transplanted HSCs mature and recover normal function. The components of the HCT process are listed below.

The hematopoietic cell transplantation recipient

Indications for hematopoietic cell transplantation

HCT is performed for a variety of malignant and nonmalignant hematologic disorders. The most common indications for HCT are summarized in Table 15-1.

Transplant eligibility

Having a condition amenable to treatment with HCT is not enough for a patient to be eligible for transplant. Transplant eligibility is determined by a comprehensive pretransplant evaluation, which includes assessment of comorbidities, organ function, and psychosocial factors to estimate the ability of a patient to tolerate transplantation and the risk-benefit of HCT compared with less toxic treatment approaches. Table 15-2 summarizes the most commonly used criteria to determine HCT eligibility.

Donors for hematopoietic cell transplantation

HCT traditionally has been classified according to the source of hematopoietic cells as either autologous or allogeneic. Allogeneic donors may be matched sibling (related),

Table 15-1 Most common indications for HCT

Autologous HCT		Allogeneic HCT	
Diagnosis	No. performed in the United States, 2015	Diagnosis	No. performed in the United States, 2015
Multiple myeloma	7,400	Acute myeloid leukemia	3,200
Non-Hodgkin lymphomas	3,000	Acute lymphoblastic leukemia	1,300
Hodgkin lymphoma	900	Myelodysplastic syndromes	1,100
		Non-Hodgkin lymphoma	770
		Myeloproliferative neoplasms	540
		Severe aplastic anemia	340

matched unrelated, mismatched unrelated, haploidentical (relative sharing 1 HLA haplotype), or unrelated newborns via umbilical cord blood. In addition, hematopoietic cells for transplantation may come from either harvested bone marrow, mobilized peripheral blood, or cord blood.

Autologous hematopoietic cell transplantation

Autologous HCT uses hematopoietic stem cells obtained from the patient, who is both the donor and the recipient of these cells. The stem cells can be obtained directly from the patient's marrow through bone marrow aspiration or through mobilization of stem cells from the marrow into the peripheral blood using high doses of the cytokine G-CSF. Stem cell mobilization can be facilitated by the administration of chemotherapy prior to G-CSF or the concurrent administration of a CXCR4 antagonist (eg, plerixafor) with G-CSF.

Allogeneic hematopoietic cell transplantation

Allogeneic HCT involves using hematopoietic cells obtained from a third party who can be a related or unrelated donor.

For some diseases, the choice between autologous vs allogeneic HCT can be difficult. In general, diseases that affect the marrow or are difficult to cure with chemotherapy alone (eg, severe aplastic anemia, acute and chronic leukemia, genetic disorders) require an allogeneic stem cell source to rescue the patient from the effects of the conditioning regimen and provide the vehicle for immunotherapy (donor lymphocytes). In diseases in which a steep dose-response curve to alkylating agents is observed and the role of a GVT effect is less certain (eg, lymphoma, myeloma, and germ cell tumor) the use of autologous stem cells is generally preferred, at least for first transplant. The use of gene therapy for genetic disorders such as

hemoglobinopathies, posttransplant maintenance therapies, and powerful targeted immunotherapies such as bispecific T cell-engaging antibodies and chimeric antigen receptor modified T cells may change some of these traditional uses of allogeneic HCT over autologous HCT.

Human leukocyte antigen typing

The ideal allogeneic donor is identified according to HLA compatibility as determined by HLA typing. The major histocompatibility complex (MHC) refers to the entire genetic region containing the genes encoding tissue HLA antigens. In humans, the MHC region lies on the short arm of chromosome 6 and is designated the HLA region. The HLA region is a relatively large section of chromosome 6 with many genes, not all of which are involved in immune responses. The HLA region has been divided into class I, class II, and class III regions, each containing numerous gene loci that may encode a large number of polymorphic alleles.

Class I antigens are composed of 2 chains: a heavy chain containing the polymorphic region that combines with the nonpolymorphic light chain, β_2 -microglobulin, to form the final molecule. The class I HLA antigens include HLA-A, -B, and -C antigens and are expressed on almost all cells of the body at varying densities. Class II antigens are composed of 2 polymorphic chains, an α chain and a β chain, which account for the majority of polymorphism in class II (both encoded on chromosome 6). The class II antigens are further divided into DR, DQ, and DP antigens. The DQ and DP antigens each have polymorphic α and β chains, whereas DR antigens contain an invariant α chain and polymorphic β chains. Class II antigens are expressed on B cells and monocytes and can be induced on many other cell types following inflammation or injury.

Table 15-2 Commonly used eligibility criteria for HCT

Eligibility criteria	Test	Transplant eligible	Comments
Patient performance status	Medical history	ECOG performance status 0–2, Karnofsky performance status >70%	Transplant mortality increases with decreasing pre-HCT performance status. Patients with poor performance status generally are not considered candidates for HCT.
Disease and disease status	Multiple	Depending on disease, disease risk and disease status. High-risk disease and high-risk disease status predict <10% 2-year survival	Patients with advanced refractory disease are generally not transplant eligible. Armand et al (2012) proposed a disease and disease status risk classification for HCT.
Infectious disease markers	Serologies for hepatitis A, B, and C. PCR for viral copies HIV, HTLV-1, CMV, EBV, toxoplasmosis	Generally, patients should not have documentation of active viral replication	Guidelines changing with the advent of effective antiviral therapy for HIV, HBV, and HCV. Prior hepatitis exposure does not affect transplant outcomes.
Cardiac function	Echocardiogram Nuclear medicine testing	Ejection fraction >40% No uncontrolled cardiac disease	Patients with cardiac disease may require more extensive pretransplant evaluation, including referral to cardiology for stress testing or Holter monitoring.
Pulmonary function	Pulmonary function testing	DLCO >40%	In some series, the most important predictor of outcome is DLCO <40%.
Renal function	Creatinine and creatinine clearance	Creatinine clearance >40 cc/min	Patients with poor renal function can be considered for HCT. Autologous HCT is performed for patients with multiple myeloma on dialysis.
Hepatic function	Liver function tests (transaminases and bilirubin)	Bilirubin <2–3 × ULN unless Gilbert disease	Elevated liver function tests predict liver toxicity.
Comorbidity scoring	Hematopoietic cell transplantation specific comorbidity indices	No cutoff determined. Poor risk categories predict increased treatment-related mortality	Comorbidity scoring is useful to guide regimen intensity and for estimation of transplant-related mortality. HCT-CI most commonly used scoring system.
Psychosocial	Various	Varies by institution	Essential to determine risk of noncompliance, substance abuse, caregiver availability, and social support needed throughout the transplant process.

ECOG, Eastern Cooperative Oncology Group; HTLV-1, human T-lymphotropic virus 1; ESRD, end-stage renal disease; ULN, upper limit of normal; HCT-CI, hematopoietic cell transplantation-comorbidity index.

Determination of HLA types has become refined over time as typing has become molecularly based, replacing the earlier serologic or cellular techniques. Modern HLA typing relies on molecular techniques, such as polymerase chain reaction (PCR) amplification of the test DNA followed by probing with labeled short sequence-specific oligonucleotide probes or, more recently, sequencing of the MHC class I and class II alleles. By convention, differences recognized by serologic typing are called antigen mismatches, and differences recognized only by molecular techniques are called allele mismatches.

Matched related and unrelated donors

Inheritance of HLA antigens is determined by Mendelian genetics with coexpression of the maternal and paternal alleles; the likelihood of siblings sharing both HLA hap-

lotypes (ie, a particular sequence of HLA-A, -B, -C, -DR, -DQ, and -DP on chromosome 6) is approximately 25%, and the chances of finding a sibling donor increases with the number of siblings in the family. Parents share 1 HLA haplotype with their offspring and are considered haploid-identical. Certain HLA antigens commonly occur in association with one another, a phenomenon called linkage disequilibrium. This limits the number of potential HLA haplotypes that occur and allows for the development of large feasible donor registries.

For the majority of patients who lack a matched related donor (MRD), an HLA-identical unrelated donor represents an alternative stem cell source. Millions of potential donors have been HLA typed and are listed in national and international registries. Because of linkage disequilibrium (nonrandom association of HLA alleles) with a high

frequency of HLA allele association due to the common location of HLA genes on chromosome 6, common haplotypes are found more frequently within the registry. Thus, for patients with common HLA types, it is now possible to find donors on a routine basis. It is still difficult, however, to find a donor for patients with infrequent haplotypes or for patients with polymorphic HLA backgrounds, such as African Americans or those of mixed race.

Cord blood transplantation and haploidentical donor transplantation

Because of the inability to identify a matched related or unrelated donor for all patients in need of an allogeneic HCT, additional sources of stem cells have been explored. It is estimated that a quarter to a third of patients in need of an unrelated donor are not able to find a match. Therefore, umbilical cord blood (UCB) cells harvested from the umbilical cord of newborns represent an alternative source of HSCs. UCB contains hematopoietic progenitors capable of hematopoietic reconstitution, can be obtained within a short time span (available on average in 2 weeks in contrast to a matched unrelated donor [MUD] search of 3 to 4 months), and demonstrates less allogeneic reactivity responsible for GVHD compared with marrow or peripheral blood grafts. Because of the relative immaturity of the newborn immune system, cord blood transplantation can be performed with a relatively low incidence of GVHD even with 2 and 3 HLA antigen mismatches.

The greatest limitations of UCB transplantation are slow engraftment with prolonged cytopenias, engraftment failure, and delayed immune reconstitution that results in higher rates of death from infection. All of these limitations are related to the relatively low progenitor cell dose in cord blood units. This low progenitor cell dose has hampered the ability to obtain rapid engraftment in patients who weigh >50 kg. Techniques to improve engraftment of umbilical cord stem cells are actively studied in clinical trials and include ex vivo expansion of cord blood HSCs and improving homing of HSCs to the bone marrow niches. Ex vivo expansion of cord blood HSCs can be accomplished through blockade of stem cell differentiation with agents such as the SIRT1 inhibitor nicotinamide, through aryl hydrocarbon receptor antagonism with the purine derivative SR1, or with the pyrimidodine derivative UM171. Promotion of homing of cord blood HSCs to the bone marrow niches can be accomplished with direct intrabone injection, dipeptidyl peptidase 4 inhibition with sitagliptin, CXCR4 activation with complement fragment 3a, treatment with dimethyl PGE2, and enforced HSC fucosylation to enhance interaction of HSCs with selectins on marrow endothelial cells.

Clinical trials of UCB transplantation in adults have shown prolonged time to engraftment with a median time to neutrophil engraftment of 3 to 4 weeks, with up to 10% of patients failing to engraft. An analysis from the International Bone Marrow Transplant Registry showed that the results with UCB transplantation were equivalent to those of mismatched unrelated-donor transplantation but were inferior to matched unrelated-donor transplantation. Attempts to hasten engraftment have included the use of 2 cord blood units in a single patient, supplementation with CD34 selected cells from a related donor, and the use of ex vivo partially expanded UCB products. Of particular interest is the observation that cord blood transplantation may be associated with lower rates of relapse than other products, potentially because of the coinfusion of maternal cells.

Another potential source of stem cells for patients without an HLA identical donor within their families or from volunteer donor registries is mismatched family members sharing 1 HLA haplotype. Donors that share 1 haplotype with the recipient are called haploidentical. Parents and their children are HLA haploidentical with each other and siblings have a 50% chance of being haploidentical with each other. It is estimated that approximately 90% of patients have a haploidentical donor. The major challenges associated with haploidentical transplantation are severe acute GVHD and delayed immune reconstitution. Strategies to ameliorate GVHD include T cell depletion both through in vivo and ex vivo means, novel immunosuppressive combinations, and posttransplantation chemotherapy with cyclophosphamide. Graft failure has been reduced with the use of large doses of stem cells and intensified conditioning regimens.

Retrospective comparisons of transplant outcomes have shown similar results for recipients of haploidentical and cord blood transplants. Trials currently are under way to determine whether there is an optimal alternative stem cell source for patients lacking an HLA-compatible donor within their family or the unrelated donor registries. In addition, for some diseases haploidentical transplantation may yield similar outcomes to traditional allogeneic donor HCT. Given that haploidentical donors can be rapidly identified, the speed with which a haploidentical transplant can be performed may make it preferable to a matched unrelated-donor transplantation in select patients.

Hematopoietic stem cell sources and procurement

HSCs reside primarily in the bone marrow but circulate in the peripheral blood at low levels. Chemotherapy, G-CSFs, and the CXCR4 inhibitor plerixafor can mobilize

large quantities of HSCs into the peripheral blood for subsequent collection via leukapheresis. Early in the history of marrow transplantation, marrow was infused fresh after collection with minimal manipulation (filtering of fat globules and bone particles, plasma and/or red cell reduction, depending on ABO incompatibility). With the advent of the cryopreservation agent dimethyl sulfoxide, cryopreservation of marrow or peripheral blood HSCs became feasible and was adopted rapidly for autologous marrow and peripheral blood stem cell (PBSC) harvesting and to a lesser degree for cryopreservation of allogeneic marrow or PBSC.

Bone marrow

HSCs initially were obtained exclusively from the marrow cavity under anesthesia with multiple aspirations by a procedure first described in the 1950s. In the setting of marrow transplantation, stem cell dose has been identified as an important predictor of outcome, with patients receiving larger stem cell dose having more rapid engraftment, reduced nonrelapse mortality (NRM), and improved survival.

Peripheral blood

The discovery that peripheral blood contained low levels of circulating hematopoietic pluripotent progenitor cells was made in the 1970s. The subsequent cloning and clinical development of colony-stimulating factors allowed for mobilization of large numbers of HSCs into the peripheral blood for collection by leukapheresis. Collection from the blood obviated the need for bone marrow harvesting and made HCT with PBSCs feasible for large-scale study and use.

Under steady-state conditions, most HSCs reside in the marrow, and various strategies have been developed to mobilize them into the bloodstream. This includes single-agent cytokine (typically G-CSF), cytokine combinations, and combinations of chemotherapy with cytokines followed by collection of peripheral blood leukocytes with leukapheresis. HSC concentration in the bloodstream usually peaks 4 to 6 days after initiation of therapy with cytokines alone. When chemotherapy with cytokines (eg, G-CSF) is given, maximum recovery of stem cells in the blood occurs at the time of marrow recovery. Collection usually is initiated when the white blood cell (WBC) count recovers to $>1 \times 10^9$ WBC/L. To improve the accuracy and efficacy of stem cell collections, daily measurement of peripheral blood CD34⁺ cell content has been used, and many centers initiate HSC collection when CD34⁺ cell counts exceed 5 to 10 cells/ μ L.

Mobilized peripheral blood hematopoietic cells have almost completely replaced bone marrow as the HSC

source for patients undergoing autologous HCT because of the less invasive collection method and more rapid blood count recovery. The more rapid recovery is thought to be due to higher stem cell doses infused with PBSCs. In the autograft, increasing stem cell dose is associated with more rapid platelet and neutrophil recovery when stem cell doses of 2 to 10 million CD34⁺ cells/kg are used. CD34⁺ cell doses lower than 2 million CD34⁺ cells/kg compromise the efficiency and success of engraftment.

Despite the use of chemotherapy–cytokine combination regimens, mobilization failure still occurs in some patients needing an autologous HCT. Prior chemotherapy and/or radiation treatment is the single most important factor affecting stem cell yields. Prior treatment with stem cell toxins, short interval since last chemotherapy, previous radiation, hypocellular marrow at collection, malignancies involving the bone marrow, and refractory disease have been associated with poor mobilization. This underscores the importance of referring a potential transplantation candidate early for autologous transplantation evaluation before repeated salvage chemotherapy attempts that may adversely affect stem cell collections.

A small molecule CXCR4 inhibitor, plerixafor, was approved in 2009 as a mobilization agent in combination with G-CSF. Plerixafor, a bicyclam derivative, is a specific antagonist of CXCR4, a coreceptor for the entry of HIV into host cells and initially was developed as a potential therapeutic agent for HIV. In a phase 1 study, it induced modest leukocytosis when administered intravenously to HIV-infected patients. On the basis of this observation, plerixafor was tested for its ability to mobilize CD34⁺ cells and hematopoietic progenitor cells from the marrow into the peripheral blood. In a pilot study, plerixafor caused a rapid and significant increase in the total WBC and peripheral blood CD34⁺ counts at 4 and 6 hours after a single injection. The results of 2 phase 3 randomized studies, 1 involving lymphoma patients and the other myeloma patients, have been completed. Patients were randomized to receive G-CSF alone or G-CSF in combination with plerixafor. In both studies, a significantly higher proportion of patients in the G-CSF–plerixafor arm collected adequate stem cells compared with the G-CSF alone arm. Plerixafor was well tolerated, and the most common adverse events were gastrointestinal (GI) disorders (eg, diarrhea) and injection site reactions. In the autologous transplant setting, peripheral blood HCT has almost totally replaced bone marrow as a source of stem cells because of the ability to collect a large number of stem cells in a less invasive manner with a stem cell product that results in a more rapid engraftment and reduction in complications.

Compared with bone marrow in allogeneic HCT, peripheral blood hematopoietic cells are associated with faster engraftment and less failure to engraft but at the expense of higher rates of chronic GVHD. Nine randomized trials have been performed comparing peripheral blood vs bone marrow in the setting of matched related-donor transplantation. In a meta-analysis of individual data of these trials, peripheral blood led to faster neutrophil and platelet engraftment and was associated with a significant increase in the development of grade 3 to 4 acute GVHD and extensive chronic GVHD at 3 years. Peripheral blood also was associated with a decrease in relapse (21% vs 27% at 3 years) both for advanced and early stage hematologic malignancies. Peripheral blood was not associated with lower rates of NRM; however, in patients with advanced disease, it was associated with improvements in overall and disease-free survival (DFS).

In children, the increased risk of chronic GVHD has led to the use of bone marrow as the preferred source of stem cells. T cell depletion with CD34 selection has been used to reduce the increased risk of chronic GVHD but further prospective trials are needed.

Umbilical cord blood

Broxmeyer et al were the first to report the presence of HSCs in umbilical cord blood using the granulocyte-macrophage progenitor cell assay. They were also the first to find that procedures to remove erythrocytes or granulocytes before freezing and washing of thawed cells before plating entailed large losses of progenitor cells. These findings laid the foundation for current umbilical cord blood banking. This ultimately led to the first successful cord blood transplant in a young patient with Fanconi anemia. The low HSC content in cord blood has posed a challenge for use in adults. Strategies such as use of 2 cord blood units in a single patient, however, now enable routine use of UCB transplantation in adults as well. Novel technologies to expand progenitor cells in cord blood units ex vivo or improve stem cell homing to the bone marrow may further broaden access to this cell source for adult transplantation.

Conditioning regimens

The combination of chemotherapeutic and physical agents given prior to HCT is known as the conditioning or preparative regimen. The purpose of conditioning in both the autograft and allograft setting is to eradicate the malignancy with high-dose chemotherapy or radiation therapy. In the setting of allogeneic HCT, the conditioning regimen also suppresses the recipient's immune system to prevent rejection of donor hematopoietic cells.

The more immunosuppressive the conditioning regimen is to the host, the better the chance for engraftment. Conditioning regimen intensity is classified according to myelosuppressive effects into the categories of fully myeloablative, reduced intensity, and nonmyeloablative. Table 15-3 lists the most commonly used conditioning regimens currently in use.

Myeloablative regimens

The first conditioning regimen that achieved widespread application consisted of the combination of cyclophosphamide and total body irradiation (CyTBI). High doses of cyclophosphamide, typically 120 to 200 mg/kg, are combined with radiation in a dose of 8 to 12 Gy (depending on the fractionation). This regimen is myeloablative and profoundly immunosuppressive. High-dose busulfan and cyclophosphamide (BuCy) conditioning was developed as an alternative to CyTBI. Treatment-related morbidity and mortality rates are similar after both regimens, although the patterns of toxicity are slightly different. TBI is associated with more pulmonary toxicity, cataract formation, and thyroid dysfunction. BuCy is associated with a higher incidence of sinusoidal obstruction syndrome of the liver (SOS; formerly veno-occlusive disease [VOD]) and irreversible alopecia. Fludarabine/busulfan combinations have become increasingly utilized because cyclophosphamide and its metabolites increase the risk of SOS. Busulfan-based protocols generally are recommended young children because of the long-term deleterious TBI.

Nonmyeloablative and reduced-intensity conditioning

Myeloablative conditioning regimens were long considered necessary for engraftment of allografts, but their considerable extramedullary toxicity typically limited their use to patients <50 to 60 years of age who had a good performance status and no comorbidities. The demonstration that engraftment can be achieved without myeloablation led to the investigation of nonmyeloablative (NMA) and reduced-intensity conditioning (RIC) regimens. These regimens often use lower doses of busulfan, melphalan, cyclophosphamide, or TBI (typically 2 Gy), often in combination with fludarabine for immune suppression. Nonmyeloablative and reduced-intensity conditioning regimens are more frequently used in older patients, in patients with comorbidities, and in nonmalignant bone marrow disorders. For malignant conditions, these regimens rely heavily on immunologic (GVT or graft-versus-leukemia [GVL]) effects to achieve long-term remissions and contain lower doses of drugs with cytoreductive activity. Although treatment-related deaths are less frequent with NMA/RIC regimens compared with myeloablative

Table 15-3 Commonly used conditioning regimens

Allogeneic hematopoietic cell transplantation	
Myeloablative conditioning	
Cy TBI	Cyclophosphamide 120 mg/kg+TBI 8–12 Gy*
Bu Cy	Cyclophosphamide 120 mg/kg + busulfan 9.6–12.8 mg/kg IV or PO equivalent
Flu Bu	Fludarabine 120–150 mg/m ² + busulfan 9.6–12.8 mg/kg IV or PO equivalent
Reduced-intensity conditioning	
Flu Mel	Fludarabine + melphalan 140 mg/m ²
Flu Bu	Fludarabine + busulfan 6.4 mg/kg IV or PO equivalent
Nonmyeloablative conditioning	
Flu TBI	Fludarabine + TBI 2 Gy
Flu Cy	Fludarabine + cyclophosphamide 60 mg/kg
Cy ATG	Cyclophosphamide 4 g/m ² +ATG†
Autologous hematopoietic cell transplantation (all myeloablative)	
Lymphoma	
BEAM	BCNU + etoposide + cytarabine + melphalan
BEAC	BCNU + etoposide + cytarabine + cyclophosphamide
CBV	Cyclophosphamide + BCNU + etoposide
Myeloma	
High-dose melphalan	Melphalan 200 mg/m ²

*Various fractionation schedules in use.

†Mainly for conditioning in severe aplastic anemia.

regimens, GVHD and infections remain the major causes of NRM.

For a reduced-intensity conditioning regimen, low nonhematologic toxicities and some degree of mixed chimerism early posttransplant is desired. For NMA regimens, they theoretically could be given without stem cell support. Operationally, reduced-intensity conditioning regimens have been defined by the following doses of commonly administered agents: melphalan <150 mg/m²; busulfan <9 mg/kg of the oral equivalent; thiotapec <10 mg/kg; and TBI <500 cGy single fraction or 800 cGy fractionated. These definitions are somewhat arbitrary but are important for retrospective studies.

The optimal conditioning intensity for patients for malignant hematologic disorders remains a matter of ongoing debate. Retrospective analyses have yielded conflicting results. Several randomized trials have been performed to address this question. To date, reported results are conflicting, but the trial sizes have been relatively small. The largest trial to date performed by the Blood and Marrow Transplant Clinical Trials Network randomized patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS) to RIC or fully myeloablative conditioning allogeneic HCT. The study was

stopped early because of a high relapse rate in the RIC arm. Overall survival (OS) at 18 months was 68% in the RIC arm vs 78% in the myeloablative arm ($P=0.07$). TRM was 4.4% for RIC vs 16% with myeloablative conditioning (MAC) ($P=0.002$), whereas relapse-free survival was 47% with RIC vs 68% with myeloablative conditioning (MAC) ($P<.01$). Based on these results, myeloablative conditioning may be preferred for fit adult patients with AML or MDS.

Regimens for autologous hematopoietic cell transplantation

Given the lack of GVT effect with autologous transplantation, all regimens are myeloablative to attempt to cure or control disease with high-dose chemotherapy. Autologous HCT conditioning regimens are used nearly exclusively for autologous HCT. Regimens include: (1) carmustine-based regimens, such as carmustine, etoposide, cytarabine, and melphalan (BEAM), or cyclophosphamide, carmustine, and etoposide for relapsed/refractory non-Hodgkin lymphomas (NHLs) or Hodgkin lymphoma (HL); (2) high-dose melphalan regimens used for multiple myeloma; and (3) the carboplatin and etoposide regimens for relapsed germ cell tumors.

Conditioning for benign hematologic disorders

Patients with aplastic anemia, metabolic disorders, or hemoglobinopathies represent a special category. There is no underlying malignancy that requires eradication. There is a higher risk of graft rejection, in part because of the nature of the underlying disease, the lack of previous immunosuppressive chemotherapy and, in many cases, exposure to prior transfusions with HLA sensitization. Thus, the conditioning regimens for such patients traditionally have emphasized more immunosuppression and less myelosuppression. A combination of high-dose cyclophosphamide with antithymocyte globulin (ATG) has emerged as the standard conditioning regimen for aplastic anemia. Conditioning therapy is more challenging for patients with Fanconi anemia because of excessive toxicity of cyclophosphamide in these patients.

Phases of hematopoietic cell transplantation

Successful HCT requires the patient to tolerate the conditioning regimen, HSCs to engraft, proliferate, and mature normally, adequate prevention and treatment of infectious complications related to myelosuppression and immunosuppression in the first months after HCT, and, in the case of allogeneic HCT, prevention and treatment of GVHD. Given the complexity and unique complication of HCT, most HCTs in North America are performed at specialized centers with teams of physicians, nurses, and other personnel dedicated to the care of patients undergoing HCT. Outcomes are improved when HCTs are performed in specialized transplant units that perform a minimum of at least 10 transplants a year.

The HCT procedure can be divided into 5 phases, as detailed below and summarized in Table 15-4.

Phase I: conditioning (day –10 to day 0)

During this phase, chemotherapy (usually at high doses) with or without radiation is given to the patient to eliminate any residual malignant cells, provide physical space for the donor stem cells and, in the case of allogeneic HCT, suppress the recipient immune system to facilitate donor cell engraftment. Phase I finishes with the infusion of the hematopoietic cells provided either by the patient in the case of an autologous HCT or by a donor in the case of an allogeneic HCT.

Phase II: cytopenic phase (day 0 to engraftment)

The most obvious effects of the high doses of chemotherapy and radiation therapy are felt during this phase. Severe

myelosuppression and disruption of the GI mucosa manifested as stomatitis and diarrhea during this period can last 10 to 28 days. During this period, serious infections and organ toxicities such as sinusoidal occlusion syndrome of the liver and idiopathic pneumonia syndrome can occur.

Phase III: early recovery (engraftment + 7 days)

In this initial phase of neutrophil recovery, patients can develop a syndrome characterized by fever, rash, and pulmonary infiltrates known as the “engraftment syndrome,” which, when identified, should be treated promptly with corticosteroids. This period also marks the most common time when acute GVHD can begin to manifest in the allograft setting.

Phase IV: early convalescence (day +30 to 6 to 12 months)

This phase is characterized by persistent immune deficiency despite normal peripheral blood cell counts. Patients remain at risk of serious life-threatening opportunistic infections that require antibacterial, antiviral, and antifungal prophylaxis as well as close monitoring for infection by the transplant team. Patients undergoing allogeneic HCT continue to be at risk for acute as well as chronic GVHD. Late organ side effects may arise, especially lung toxicities, including pneumonitis from conditioning, cryptogenic organizing pneumonia, and bronchiolitis obliterans syndrome (BOS). Relapse risk is highest in the first year after transplant. Posttransplant lymphoproliferative disorder (PTLD) driven by Epstein-Barr virus (EBV) may develop during this time.

Phase V: late convalescence (beyond 12 months)

This final phase is characterized by the almost full recovery of the immune system and by the potential of late complications, such as organ dysfunction, cataracts, secondary malignancies, or recurrence of the original malignancy. Patients undergoing allogeneic HCT are at ongoing risk of developing chronic GVHD.

Hematopoietic cell transplantation complications

Bone marrow and immune system toxicities

Myelosuppression

Myelosuppression is a universal complication of myeloablative conditioning regimens. The duration of the myelosuppression depends on various factors, including the hematopoietic stem cell dose, use of methotrexate as GVHD prophylaxis, extent of prior therapy, and stem cell source

Table 15-4 HCT complications according to transplant phase

	Phase I: conditioning	Phase II: cytopenic phase	Phase III: early recovery	Phase IV: early convalescence	Phase V: late convalescence
Timing	Day -10 to D0	D0 to engraftment	Engraftment +7d	D+30 to 6–12 months	>12 months
Infections	Catheter-related	GPC, GNR from GI mucosal toxicity HSV Fungal infections Catheter-related	Resistant GNR or GPC Fungal infections CMV reactivation EBV reactivation Other viruses	Viral reactivations <i>Pneumocystis</i> Encapsulated GPC EBV+ PTLD	Viral reactivation (if active GVHD) Encapsulated GPC
Gastrointestinal	Nausea and vomiting Diarrhea	Mucositis Diarrhea Nausea Anorexia	Protracted nausea and/or anorexia can be sign of upper GI GVHD	Gut GVHD: diarrhea, abdominal pain, nausea, anorexia	
Hepatic	Transaminitis	Transaminitis Sinusoidal obstruction syndrome	Transaminitis Sinusoidal obstruction syndrome Liver GVHD	Hepatitis virus reactivation Liver GVHD	Cirrhosis
Cardiac	Arrhythmias (rare) Fluid overload	Hypertension from CNI	Hypertension from CNI	Hypertension from CNI	Congestive heart failure Premature coronary vascular disease
Pulmonary	Pneumonitis (rare)	Infectious pneumonia Fluid overload Idiopathic pneumonia syndrome	Infectious pneumonia Idiopathic pneumonia syndrome Diffuse alveolar hemorrhage	Cryptogenic organizing Infectious pneumonia	Bronchiolitis obliterans syndrome Hyperactive airway disease Infectious pneumonia
Neurologic	Seizures from busulfan (rare with prophylaxis)	PRES (from CNI)	PRES (from CNI)	PRES (from CNI)	Cognitive dysfunction—short-term memory loss Impaired concentration
Endocrine	Hyperglycemia	Hyperglycemia from CNI	Hyperglycemia from CNI	Hyperglycemia Hypothyroidism	Metabolic syndrome
Renal	Increased creatinine Electrolyte abnormalities	Increased creatinine due to drugs (antibiotics, antifungals, CNI) Electrolyte disturbances	Increased creatinine Electrolyte disturbances	Chronic renal failure	Chronic renal failure
Acute graft-versus-host disease			Initial presentation can be rash and fevers	Late acute GVHD presents as acute onset diarrhea, rash, transaminitis, or hyperbilirubinemia	
Chronic graft-versus-host disease				Usually presents in the context of immune suppression withdrawal	Usually presents in the context of immune suppression withdrawal
Other				PTLD	Cataracts Secondary malignancies

GPC, gram-positive cocci; GNR, gram-negative rods; CNI, calcineurin inhibitor.

(peripheral blood vs bone marrow aspirate vs umbilical cord blood). Engraftment is defined as sustained recovery of an absolute neutrophil count of $>500/\mu\text{L}$ and unsupported platelets $>20,000/\mu\text{L}$ for 3 consecutive days. In the context of an allogeneic HCT, this also implies evidence of donor cell engraftment. Filgrastim has been shown to reduce the time to neutrophil engraftment in both the autologous and allogeneic setting but without definitive improvement in HCT outcomes such as OS.

Graft failure

Graft failure is an unusual but often fatal complication of HCT. Mechanisms include immunologic rejection, abnormalities in the marrow microenvironment or stroma, inadequate dose or composition of the graft, viral infections (in particular cytomegalovirus [CMV]), or drug-induced myelosuppression. It often is impossible to determine the exact cause of graft failure in an individual patient, but the risk for graft failure is increased with increasing HLA disparity between the graft and host, with T cell depletion of the graft, the use of bone marrow or cord blood as a stem cell source, and in transplantation for certain diseases, such as severe aplastic anemia or hemoglobinopathies. The risk for graft rejection can be decreased by infusing larger numbers of HSCs and by increasing the intensity of the conditioning regimen. Successful treatment of graft failure usually involves reinfusing more stem cells either from the original stem cell donor or another source if the original donor is unavailable. Graft failure after autologous HCT is rare but can happen because of infections or toxic drug exposure. Heavily pretreated patients receiving suboptimal doses of stem cells (<2 million CD34 $^+$ cells/kg) frequently have poor graft function after autologous HCT and have a higher rate of developing secondary MDS or AML.

Infection

Infections are a major cause of life-threatening complications in HCT. Their prevention, diagnosis, and treatment are important components of the care of the HCT patient. Major advances in this area have decreased treatment-related mortality. Although this is an ever-changing field, the Centers for Disease Control and Prevention recommendations published in 2000 and updated in 2009 provide an essential framework for treatment and prevention.

Bacterial infections commonly occur during the neutropenic period after transplantation, and guidelines for their prevention and management are similar to those in other neutropenic patients. The use of prophylactic fluoroquinolone antibiotics is standard for patients older than 12 years of age during the neutropenic period. The American Academy of Pediatrics currently recommends

that fluoroquinolones be limited in children to a number of circumstances that include gram-negative bacteremia in the immunocompromised host in which an oral agent is desired. As the experience with fluoroquinolones in young children grows, there is likely to be an analysis of their benefit in this age group. Patients with chronic GVHD are immunosuppressed by therapy for GVHD as well as GVHD itself. They are at particular risk for fulminant infections with encapsulated gram-positive organisms, particularly *Pneumococcus*. They should receive prophylaxis with penicillin V potassium.

HCT patients are at high risk for *Pneumocystis jirovecii* (formerly known as *Pneumocystis carinii*) pneumonia and prophylaxis is recommended. Trimethoprim-sulfamethoxazole is the preferred prophylactic drug. For those allergic to trimethoprim-sulfamethoxazole, alternatives such as pentamidine, atovaquone, or dapsone are commonly used. Trimethoprim-sulfamethoxazole prophylaxis also may prevent toxoplasmosis, which occasionally has been reported in recipients of allogeneic transplantation.

Fungal infections remain a major problem in allogeneic transplantation patients and are associated with prolonged neutropenia, immunosuppression, and GVHD. Yeast (*Candida*) infections are rare with fluconazole prophylaxis. In this setting, candida infections are typically caused by fluconazole-resistant organisms. Airborne molds, particularly *Aspergillus* species, remain a major hazard for patients undergoing allogeneic transplantation, despite the use of high-efficiency particulate air filtration. The azoles (eg, voriconazole, posaconazole, isovuconazole) and echinocandins (eg, caspofungin, micafungin, anidulafungin) with potent activity against molds have improved the outcome for such patients. Broad-spectrum azoles such as posaconazole and isovuconazole are now routinely used for prophylaxis of fungal infections in patients undergoing HCT and those receiving higher doses of systemic corticosteroids for GVHD therapy. Concerns with new azoles include their toxicity profile (neurologic and hepatic toxicity). Interactions with the metabolism of calcineurin inhibitors warrant the need for careful monitoring and often dose reduction of tacrolimus and cyclosporine. Also, because *Aspergillus* species are treated more successfully, cases of mucormycosis increasingly are reported and necessitate treatment with amphotericin derivatives or newer-generation azoles such as posaconazole and isovuconazole.

Viral infections are common after HCT. CMV infection used to be a major cause of pneumonia and death in HCT recipients. CMV infection post-HCT usually occurs as a consequence of CMV reactivation in patients previously exposed to CMV as indicated by positive antibody titers (CMV $^+$ patients) prior to transplant. The incidence

of reactivation ranges from 40% to 60% in the allogeneic setting and <5% in the autologous setting depending on the technology used for screening, the target tissue evaluated (eg, blood, urine, bronchoalveolar lavage [BAL]), the conditioning regimen, and the method of GVHD prophylaxis. Detection of CMV in the blood (CMV viremia), either by PCR or rapid antigen screening, indicates a high risk for development of invasive CMV disease, usually CMV pneumonia but occasionally (especially at later time points after transplantation) CMV hepatitis, retinitis, or gastroenteritis. Patients who have not been exposed before transplantation (CMV⁻) are still at risk for CMV infection either by transmission from a CMV⁺ stem cell donor or via transfusion of blood products from a CMV⁺ blood donor. To avoid risk of CMV infection in CMV⁻/⁺ donor/recipient pairs, CMV⁻ blood products formerly were recommended but often were not readily available. Fortunately, leukocyte filtration of blood products efficiently reduces the risk of CMV transmission, and most centers no longer require use of CMV⁻ blood products.

Frequent screening for CMV viremia is mandatory in the first 3 months after allogeneic (but not autologous) HCT. Ganciclovir, oral valganciclovir, high-dose acyclovir, and valacyclovir have all been used for prophylaxis of CMV reactivation in patients at high risk, although the last 2 drugs have unproven efficacy. Each of these approaches has potential problems, including cost, inconvenience, and adverse effects. Myelosuppression, especially neutropenia, is the most serious and common toxicity associated with ganciclovir and valganciclovir, leading most providers to take a preemptive vs prophylactic use of these drugs to limit invasive CMV disease. Letermovir is a novel anti-viral that inhibits the CMV-terminase complex. A phase 3 randomized study of letermovir vs placebo after allogeneic HCT showed a significant reduction in clinically significant CMV infection (CMV reactivation requiring preemptive therapy or invasive CMV disease) from 61% with placebo to 38% with letermovir ($P <0.001$) without a difference in invasive CMV disease or death from any cause. Given no difference in invasive CMV disease or death between the groups, use of letermovir is currently institution dependent.

For patients who develop CMV viremia, preemptive treatment with ganciclovir or valganciclovir prior to the development of invasive CMV disease is initiated immediately. This strategy of preemptive treatment has significantly decreased the occurrence of CMV disease in the early months after transplantation. Oral valganciclovir is a convenient and effective oral alternative for preemptive and prophylactic treatment. Alternative medications for preemptive treatment include foscarnet (equally effica-

cious but nephrotoxic) and cidofovir (requires only once-weekly administration but is less extensively tested, very nephrotoxic, and myelosuppressive). Acyclovir and valacyclovir, although moderately active for CMV prevention, have no role in preemptive treatment.

Other important herpes viruses include herpes simplex virus (HSV), varicella-zoster virus (VZV), EBV, and human herpesvirus 6 (HHV-6). HSV used to be a major cause of mucositis and pneumonia occurring during the neutropenic phase after transplantation and is prevented by acyclovir. VZV can cause zoster, a frequent problem after transplantation with patients at risk for dissemination when profoundly immunosuppressed. In a single-institution, double-blind controlled trial, patients after an allogeneic transplantation who were at risk for VZV reactivation were randomized to acyclovir 800 mg twice daily or placebo given from 1 to 2 months until 1 year after transplantation. Acyclovir significantly reduced VZV infections at 1 year after transplantation (hazard ratio, 0.16; $P=0.006$). EBV can cause posttransplantation lymphoproliferative disease, particularly in patients who are extremely immunosuppressed because of mismatched or T cell-depleted transplantation. Withdrawal of immune suppression is done when possible to stimulate an immune response against EBV-infected cells. Treatment with rituximab is typically first-line therapy. HHV-6 frequently reactivates after allogeneic HCT, may cause posttransplantation encephalitis and aplasia, and is possibly linked to interstitial pneumonia and idiopathic pneumonia syndrome.

Adenovirus can cause fatal hepatitis, gastroenteritis, and pneumonitis in transplantation patients. The epidemiology and value of screening remains a matter of ongoing study. Respiratory viruses, such as respiratory syncytial virus and influenza virus, can lead to fatal pneumonias. Some centers have recommended screening of all patients during respiratory syncytial virus season and treatment with ribavirin and immunoglobulin in patients who become infected. This is, however, a controversial issue. BK virus and adenovirus have been associated with severe hemorrhagic cystitis. The frequency of infection, treatment, and value of screening are not determined.

Specific organ toxicities

Integument toxicity

Total body irradiation frequently is associated with generalized skin erythema followed by hyperpigmentation. Thiotapec is metabolized and excreted through the sweat glands around skin folds and dressings. Failure to take frequent showers and change dressings can lead to serious thiotapec skin toxicity. Likewise, patients receiving thiotapec should not use moisturizing cream during the

days immediately after receiving the drug. High-dose alkylator therapy as well as radiation is associated with alopecia, usually reversible with the occasional exception of busulfan.

Gastrointestinal toxicity

After hematopoietic cells, the GI tract is the most commonly affected organ by the conditioning regimen. As intestinal mucosa cells divide rapidly to maintain intestinal mucosal integrity, the GI tract is particularly susceptible to damage by conditioning regimens. The most common manifestations of GI toxicity are nausea, vomiting, oral lesions (stomatitis), throat pain, esophagitis, abdominal pain, and diarrhea.

Carmustine, TBI, and cyclophosphamide are highly emetogenic, whereas melphalan and busulfan are moderately emetogenic. Adequate control of nausea and vomiting requires prophylactic and therapeutic use of antiemetic medications. Acute emesis usually involves combination therapy with corticosteroids (typically dexamethasone) and 5-hydroxytryptamine type 3 (5-HT₃) receptor antagonists (eg, ondansetron, granisetron). For highly emetogenic regimens, prophylactically blocking the action of substance P with the addition of a neurokinin 1 receptor blocker (eg, aprepitant) in addition to dexamethasone and a 5-HT₃ blocker is recommended. Despite these measures, complete control of nausea and vomiting (no nausea, no emesis, and no need for breakthrough medications) is achieved in <20% of the population. Destruction of the oral and GI mucosa is a significant dose-limiting complication of high-dose therapy regimens as severe toxicity can lead to airway obstruction, severe mucosal bleeding, sepsis from intestinal flora, and intestinal perforation.

Stomatitis refers to the painful ulcerations and sores that occur on the mouths, lips, gums, and throats of patients usually 5 to 7 days after conditioning and can be seen in up to 90% of HCT recipients after myeloablative conditioning. The most important risk factor for developing severe stomatitis is the intensity of the conditioning regimen. Other factors that predict development of severe stomatitis are poor oral hygiene, extensive prior therapy, and concurrent chemoradiation. Stomatitis is a significant cause of morbidity after HCT. Studies have shown that the incidence of severe stomatitis after high-dose therapy can be reduced by recombinant keratinocyte growth factor (palifermin) in the setting of TBI and by oral application of ice chips during infusion of high-dose melphalan. Once stomatitis occurs, treatment is primarily supportive with intravenous hydration and parenteral alimentation if needed, as well as parenteral analgesics and antibiotics to prevent infections.

Diarrhea occurs in more than half of all patients receiving high-dose chemotherapy and also depends on the intensity of the conditioning. Other treatable causes of diarrhea need to be considered, particularly *Clostridium difficile* infection, antibiotic-induced diarrhea, and GVHD. Persistent diarrhea after engraftment should be investigated thoroughly with upper and lower endoscopic evaluation for tissue procurement to rule out GVHD and other treatable causes. Treatment for enteritis due to conditioning is supportive and symptomatic.

Hepatic complications

SOS/VOD of the liver is one of the most common and lethal toxicities of HCT; it occurs in 10% to 60% of patients receiving transplants, depending on both the risk factors for the patients and the vigor with which the diagnosis is pursued. SOS/VOD is caused by preparative regimen toxicity and is thought to be caused by damage to endothelial cells, sinusoids, and hepatocytes in the area surrounding terminal hepatic venules. Endothelial cells are directly sensitive to chemotherapy and radiation therapy, and cytokines released during endothelial injury also may be implicated. For instance, elevated levels of tumor necrosis factor α (TNF- α) predict development of SOS/VOD.

SOS/VOD is more common in patients with evidence of prior hepatocellular damage at the time of transplantation, heavy pretreatment before HCT, prolonged and elevated busulfan levels, or >10 to 12 Gy TBI. Other drugs, such as nitrosoureas (carmustine), also have been implicated in SOS. Prior exposure to gemtuzumab ozogamicin or inotuzumab ozogamicin significantly increases the risk of VOD, especially in those who receive the drug shortly before transplantation. Low-dose heparin and ursodiol have been used for prevention of SOS but remain controversial.

By European Bone Marrow Transplantation group (EBMT) criteria, classical SOS/VOD follows the Baltimore criteria occurring in the first 21 days after HCT and requires a bilirubin of ≥ 2 mg/dL with 2 of the following: painful hepatomegaly, weight gain $> 5\%$, or ascites. Late onset SOS/VOD occurs after 21 days and requires classical SOS/VOD findings, histologically proven SOS/VOD, or 2 or more of the classical criteria and hemodynamic or ultrasound evidence of SOS/VOD, typically decrease in velocity or reversal in portal flow. Ideally, the diagnosis should be confirmed by liver biopsy, but liver biopsy is not always possible because of the risks in critically ill patients. Treatment generally has been supportive care with judicious fluid management, salt restriction, and elimination of any potential hepatotoxic agents.

Defibrotide is an adenosine receptor agonist that increases levels of endogenous prostaglandins (PGI2 and PGE2), reduces levels of leukotriene B4, stimulates expression of thrombomodulin in endothelial cells, modulates platelet activity, and stimulates fibrinolysis by increasing endogenous tissue plasminogen activator function and decreasing the activity of plasminogen activator inhibitor 1. Defibrotide has little systemic anticoagulant activity, which is an advantage in patients with multiorgan failure. In the latest published update, 88 patients with severe SOS/VOD were treated with defibrotide. At treatment, median bilirubin was 12.6 mg/dL, and multiorgan failure was present in 97%. No severe hemorrhage or other serious toxicity was reported. Complete resolution of SOS/VOD was seen in 36%. Younger patients, those receiving autologous HCT, and those with abnormal portal flow had the highest response rates. Defibrotide is approved in Europe and the United States for the treatment of SOS.

Pulmonary toxicities

Pulmonary complications are common after HCT and some complications are associated with a high mortality. To risk stratify patients, pretransplantation evaluation includes pulmonary function tests (PFTs) and 2-dimensional echocardiogram or radionuclide ventriculography. The utility of these tests, however, is limited. A retrospective study of 1,297 HCT patients reported that decreased diffusing capacity of the lung for carbon monoxide (DLCO) and elevated alveolar-arterial partial pressure of oxygen were predictors for increased mortality. Most transplantation centers, however, do not exclude a patient from transplantation based solely on an abnormal pre-HCT PFT. Baseline reduced left-ventricular ejection fraction predicts for cardiac toxicity after HCT but does not appear to predict increased treatment-related mortality.

During the early transplantation period (days 0 to +30), regimen-related toxicity and infection account for most pulmonary events. Although most lung infiltrates are infectious, diffuse infiltrates related to regimen-related toxicity also should be considered. The differential diagnosis of diffuse infiltrates during the early HCT period includes iatrogenic volume overload, cardiogenic pulmonary edema, idiopathic pneumonia syndrome (IPS), adult respiratory distress syndrome from chemoradiotherapy injury or sepsis, and diffuse alveolar hemorrhage (DAH). Infection and cardiogenic pulmonary edema in particular need to be excluded. After engraftment, the risk of fungal and viral infection increases. Historically, CMV pneumonitis was the most common cause of diffuse infiltrates during days +30 to +150, but its incidence has decreased dramati-

cally with the use of preemptive treatment strategies for the prevention of CMV disease. During this period, opportunistic and idiopathic pneumonias dominate the pulmonary complications. Reconstitution of immune function after HCT takes 3 to 6 months or longer, especially for patients with chronic GVHD. Infectious etiologies during this phase include bacteria, fungi, viruses, *Nocardia*, mycobacteria, and *Pneumocystis jirovecii*. Furthermore, approximately 10% of patients with chronic GVHD develop BOS, a severe obstructive airflow disease that is frequently fatal.

Idiopathic pneumonia syndrome

IPS is characterized by diffuse alveolar injury often with fever, cough, dyspnea, hypoxemia, and restrictive airway physiology. Chest x-ray usually demonstrates multilobar pulmonary infiltrates. IPS requires exclusion of other causes of lung injury especially infection, cardiogenic pulmonary edema, and DAH. BAL must be negative for infectious etiologies, including bacteria, fungi, and CMV and other viral infections. The incidence of IPS is approximately 7%, with a median time to onset of 21 days and hospital mortality ranging from 30% to 70%. The risk factors for IPS include the use of TBI or carmustine-based conditioning regimens and previous exposure to bleomycin. HHV-6 reactivation commonly accompanies IPS but a causative role for HHV-6 has not been established. Treatment of IPS is mostly supportive, but high-dose corticosteroids often are given with unclear benefit. Based on laboratory results suggesting that the inflammatory cytokine TNF- α plays a role in IPS, the TNF- α blocker etanercept has been studied as an adjunct to corticosteroids with excellent survival compared with historical experience. An attempt to study etanercept in a randomized fashion was not completed due to slow patient enrollment. Corticosteroids may be beneficial in patients in whom pulmonary damage is due to carmustine pneumonitis or in those with DAH.

Diffuse alveolar hemorrhage

DAH occurs most commonly in the first weeks after HCT and presents as idiopathic pneumonia with or without hemoptysis. Unlike IPS, the classic finding on BAL is increasingly bloody returns during BAL washings. Analysis of BAL fluid usually demonstrates red blood cells, hemosiderin-laden macrophages if blood has been present for more than 2 to 3 days, and negative microbiologic studies. Treatment of DAH is largely supportive, but retrospective studies suggest that high-dose corticosteroids starting in the range of 1 gram per day of methylprednisolone are often beneficial.

Transplantation-related obstructive airway disease

Approximately 6% to 10% of patients with chronic GVHD develop chronic airway obstruction. The most common histologic finding is constrictive bronchiolitis obliterans. BOS typically presents 3 to 12 months after an allogeneic HCT with gradual onset of dyspnea, dry cough associated with occasional wheezing, and inspiratory crackles. PFTs demonstrate an obstructive airflow pattern that does not respond to bronchodilator therapy and a reduced DLCO. Thin-section computed tomographic scans reveal bronchial dilation, mosaic pattern attenuation, and evidence of air trapping on expiration. The diagnosis often is based on clinical, imaging, and spirometric findings without a tissue biopsy. There is no clearly effective treatment of patients with BOS, and treatment is directed at chronic GVHD with immunosuppressive therapy. Lung transplantation is an option for select patients.

Thrombotic microangiopathy

Transplantation-associated thrombotic microangiopathy (TA-TMA) presents as a spectrum of disease, ranging from mild microangiopathic anemia to thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome. TA-TMA occurs more commonly after allogeneic and unrelated donor HCT. TTP frequently presents with fever, neurologic symptoms, microangiopathic hemolytic anemia, thrombocytopenia, and renal impairment. In children, TMA more closely resembles atypical hemolytic uremic syndrome. In most patients, TA-TMA is related to calcineurin inhibitors (cyclosporine, tacrolimus) and responds to discontinuing the calcineurin inhibitor. Other patients have a fulminant course and a high mortality rate. Autopsy findings include arteriolar thrombosis in the kidneys. In many patients, fungal infection, sepsis, or GVHD appear to promote the microangiopathic processes. TTP outside the transplantation setting has been associated with immunoglobulin G (IgG) antibodies that block ADAMTS13, the cleaving protease of von Willebrand factor in the plasma. Unlike patients with idiopathic TTP, patients with TA-TMA have preserved ADAMTS13 levels and do not respond to plasma exchange. Increased complement pathway activation has been associated with fatal TA-TMA and blocking the complement pathway with the C5-binding antibody eculizumab has been proposed as a treatment for TA-TMA, especially those with evidence of complement pathway activation.

Neurologic toxicities

Significant neurologic toxicity complicates approximately 10 to 20% of allogeneic HCT but is rare with autologous

HCT. The majority of neurologic toxicities appear to occur within the first 100 days of transplantation. Central nervous toxicity has been associated with reduced OS and increased NRM after allogeneic HCT. The most common toxicities associated with allogeneic HCT include posterior reversible encephalopathy syndrome (PRES), ischemic stroke, transient ischemic attacks, toxic/metabolic encephalopathies, intracranial hemorrhage with subdural hematoma being most common, infection, and peripheral neuropathy. Many neurologic toxicities are associated with medications used in conditioning and the posttransplant period. Busulfan has a high risk of seizures that can be nearly eliminated with the use of seizure prophylaxis with phenytoin or alternate anticonvulsant. Fludarabine can also cause an acute, dose-dependent central nervous system (CNS) toxicity with cognitive impairment and sensory disturbances. Calcineurin inhibitors (cyclosporine, tacrolimus) used for GVHD prophylaxis can cause tremor, headache, confusion, ataxia, and, notably, PRES. Immune-mediated neurologic complications after allogeneic HCT are rare and include myasthenia gravis, neuropathies, and encephalitis.

Of the neurologic toxicities, PRES deserves special attention. PRES typically occurs in the early posttransplant period and commonly presents with headache, confusion, vision changes, and often seizures. Brain MRI typically shows T2 enhancement in the white matter of occipital lobes, although similar lesions may be seen in the cerebellum and brainstem. Calcineurin inhibitors are the typical causative agent, although PRES has been associated with exposure to etoposide and tyrosine kinase inhibitors such as sorafenib. PRES may also be seen in association with renal failure or uncontrolled hypertension. PRES typically resolves completely after withdrawal of the offending agent or treatment of the underlying cause.

Bleeding

Although all patients with thrombocytopenia are at risk for bleeding, several hemorrhagic syndromes are peculiar to transplantation. Hemorrhagic cystitis early after transplantation usually is attributed to bladder toxicity from cyclophosphamide metabolites, although this is rare with the use of mesna. Late-onset hemorrhagic cystitis is typically from viral infection with BK virus and rarely adenovirus. Hemorrhagic cystitis can be severe and require continuous bladder irrigation, diverting nephrostomy tubes, and occasionally formalin instillation until the bladder heals. As above, diffuse alveolar hemorrhage can be a serious complication of transplantation and most often is attributed to preparative regimen toxicity.

Iron overload

Iron overload has been identified as an adverse prognostic factor for children with thalassemia undergoing HCT, and there is increasing evidence that iron overload also may have deleterious effects for patients with hematologic malignancies who undergo HCT. This particular patient population often is transfused heavily before HCT and continues to require transfusions in the peritransplantation period. One red blood cell unit contains 200 to 250 mg of iron, and significant iron accumulation can occur after 10 to 20 RBC transfusions. Iron overload increases the risk of infection, SOS/VOD, and hepatic dysfunction.

Graft-versus-host disease

Acute and chronic GVHD was traditionally defined by the time of onset. Acute GVHD was defined as any GVHD occurring before day 100 after transplantation, and chronic GVHD was defined as any GVHD occurring after day 100. It is now recognized that typical features of chronic GVHD can occur before day 100 and that typical features of acute GVHD can occur after day 100. Acute and chronic GVHD are no longer defined by their time of onset but rather by their clinical features. Two subcategories of acute (classic and persistent or late onset or recurrent) and 2 subcategories of chronic GVHD (classic and overlap) are recognized.

Acute graft-versus-host disease

Acute GVHD can affect the skin, gut, and/or liver. Acute GVHD most commonly manifests as an erythematous macular rash. The rash may progress to a confluent rash, generalized erythroderma, and blistering of the skin similar to a severe burn. Gut involvement typically causes diarrhea with crampy abdominal pain but may also cause loss of appetite, nausea, and vomiting if there is upper gut involvement. Hepatic involvement may lead to hyperbilirubinemia, transaminitis, and progressive liver failure. Acute GVHD is graded by the extent of skin rash, the amount of diarrhea, and the degree of bilirubin elevation. There are several methods of grading GVHD, but all rely on the same features, and most continue to use the original Glucksberg criteria or the modified Keystone criteria. Patients with stage I disease have skin disease and a mild course. Those with stage II to IV disease have multiorgan disease, and patients with stage III or IV disease have a poor prognosis, with mortality rates >60%.

Acute GVHD was first considered a “pure” T cell-mediated disease, with cellular injury thought to be the result of infiltration of effector T cells into target tissues.

Recent immunohistochemical studies, however, demonstrate that some infiltrating cells are natural killer (NK) cells rather than mature T cells. This observation has led many investigators to consider acute GVHD as a “cytokine storm.” This model accounts for many of the observations made in GVHD. It proposes that damage to host tissues during chemotherapy and infection results in the release of inflammatory cytokines such as TNF and interleukin-1 (IL-1). These cytokines provoke increased MHC expression and upregulate other adhesion molecules that, in turn, amplify recognition of allogeneic minor HLA differences by T cells in the donor graft. The reactive donor T cells proliferate and secrete cytokines that further activate donor T cells and other inflammatory cells, including macrophages that secrete more IL-1 and TNF. This cascade eventually produces the clinical manifestations of GVHD. Factors such as gut decontamination, sterile environment, lower-dose preparative regimens, and ex vivo lymphocyte depletion of a marrow graft decrease GVHD by interrupting this cascade. Of particular interest is further elucidation of the role of CD4⁺ subpopulations in GVHD because in experimental models, the T-helper cell type 2 (Th2) subpopulation that produces IL-4 and IL-10 (in contrast to Th1 cells, which secrete IL-2 and interferon) inhibits GVHD. Allogeneic peripheral blood hematopoietic cells are relatively enriched for the Th2 population, which may account for the relatively moderate rate of acute GVHD seen after the large T cell load given with the peripheral blood.

Prevention of GVHD is more successful than treatment of GVHD. The most commonly used GVHD prophylaxis regimens combine a calcineurin inhibitor (tacrolimus, cyclosporine) with low dose methotrexate. Because of the renal and mucosal toxicities seen with these regimens, alternative prophylactic regimens are being explored. Siroimimus and mycophenolate mofetil are alternatives to methotrexate to decrease the toxicity of GVHD prophylaxis. The use of post-transplant high-dose cyclophosphamide is also being explored in randomized studies.

Other methods to prevent GVHD include depleting the graft of donor T cells, either by an in vitro procedure after procurement of hematopoietic cells or by exposure to T cell-depleting antibodies such as ATG or alemtuzumab. These strategies result in a significant reduction in acute GVHD but can result in poor engraftment, higher infection rates because of delayed immune reconstitution, post-transplant lymphoproliferative disorders, and increased risk of relapse.

Therapy for acute GVHD consists of high-dose corticosteroids, typically 1 to 2 mg/kg/day of methylprednisolone

or the equivalent, which are tapered upon obtaining a response. Calcineurin inhibitors are continued or may be restarted. Patients not responding to or experiencing recurrence of GVHD on high doses of corticosteroids (considered steroid refractory) have a very poor prognosis with 1-year survival <10% from continued acute GVHD, infection, and chronic GVHD. Other agents added in the steroid-refractory setting include mycophenolate mofetil, pentostatin, ATG, ruxolitinib, basiliximab, and monoclonal antibodies, such as infliximab, etanercept, and rituximab. The response rates are low, however, and patients typically succumb to opportunistic infection in the setting of profound immunosuppression or from progressive organ failure due to GVHD. Given the lack of proven effective options for steroid-refractory GVHD, all patients with GVHD should be encouraged to participate in clinical trials.

Chronic graft-versus-host disease

Chronic GVHD affects from 40% to 80% of long-term survivors of allogeneic HCT and can lead to long-term morbidity, disability, and diminished quality of life. Although chronic GVHD once was designated arbitrarily as any GVHD occurring after day 100, it is now recognized as a distinct disorder in which the manifestations often resemble those seen in spontaneously occurring autoimmune disorders. The diversity of the manifestations has proven a great hindrance to clinical study of chronic GVHD. A National Institutes of Health consensus conference produced working definitions for clinical and pathologic diagnosis, staging, and response criteria, as well as suggestions for supportive care, clinical trial design, and biomarkers.

Some features of acute GVHD also can be found in patients with chronic GVHD, but patients with chronic GVHD always have, in addition, other diagnostic or distinctive features. Diagnostic features of chronic GVHD are features that are sufficient to establish the diagnosis. They are summarized in Table 15-5. Diagnostic features of chronic GVHD typically involve the skin and mucosal tissues. Features include poikiloderma, lichen planus-like features, sclerotic features, and morphea-like features of the skin. Lichen-type features and hyperkeratotic plaques of the mouth also are diagnostic, as is vaginal scarring. Other diagnostic features of chronic GVHD are the development of esophageal webs and strictures, fasciitis, and joint contractures. Finally, BOS is a diagnostic feature of chronic GVHD if confirmed by biopsy.

Distinctive signs are also typical for chronic GVHD but are not by themselves considered sufficient for a diagnosis. They include depigmentation, nail loss, alopecia, xerostomia, and myositis. Features such as thrombocytopenia, eosinophilia, lymphopenia, hypo- or hypergammaglobulinemia, exocrine pancreatic insufficiency, myasthenia gravis,

cardiac conduction abnormalities, and nephrotic syndrome can occur in chronic GVHD but are not sufficient for diagnosis.

Chronic GVHD used to be scored as limited or extensive on the basis of the need for treatment. In the National Institutes of Health scoring system, chronic GVHD is classified as mild, moderate, or severe, based on the number of organs involved and the extent of involvement within each organ.

The incidence of chronic GVHD is increasing because of the older age of patients being transplanted, the predominant use of PBSCs, and the use of mismatched and unrelated donors. The greatest risk factor for development of chronic GVHD is prior acute GVHD. Chronic GVHD has been poorly studied compared with acute GVHD because most patients have returned to their home institutions by the time this complication develops. These same factors also have hindered studies of the pathophysiology of this disorder.

Therapy in patients with chronic GVHD has relied on corticosteroids after a report by the Seattle transplantation group that corticosteroids are more effective than corticosteroids plus azathioprine. A study comparing cyclosporine plus prednisone therapy with prednisone was unable to show a benefit to combination therapy other than a steroid-sparing effect and less bone damage compared with the prednisone-alone group. Other therapies currently used but not supported by randomized trials include psoralen plus ultraviolet A, extracorporeal photopheresis, pentostatin, imatinib, rituximab, and ibrutinib.

The major cause of death in patients with chronic GVHD is infection from the profound immunodeficiency associated with chronic GVHD and its therapy. Careful monitoring with antibiotic prophylaxis for encapsulated bacteria is warranted in all patients. Patients with frequent infections and low immunoglobulin levels may benefit from intravenous immunoglobulin replacement. Patients should remain on prophylaxis for viruses, *P. jirovecii* pneumonia, and fungal infections (yeasts and molds).

Late effects

As the number of long-term survivors following a transplantation increases, the need for understanding late side effects of HCT is essential both for the care of the survivors and to anticipate the needs of the group as a whole. Joint recommendations of the EBMT, the CIBMTR (Center for International Blood & Marrow Transplant Research), and the American Society of Blood and Marrow Transplantation have been published. The recommendations are based on published data and on common practice among HCT providers. At least annual evaluation for long-term survivors is

Table 15-5 Signs and symptoms of chronic GVHD

Body site	Diagnostic of chronic GVHD	Distinctive in chronic GVHD but not diagnostic	Other features*	Common to both acute and chronic GVHD
Skin	Poikiloderma Lichen planus-like sclerosis Morphea-like features Lichen sclerosis-like features	Depigmentation	Sweat impairment Ichthyosis Keratosis pilaris Hypopigmentation Hyperpigmentation	Erythema Maculopapular rash Pruritus
Nails		Dystrophy Ridging Splitting Brittleness Onycholysis Pterygium unguis Nail loss [†]		
Scalp and body hair		Scarring/nonscarring scalp alopecia Scaling Papulosquamous lesions	Thinning scalp hair Premature graying	
Mouth	Lichen-type features Hyperkeratotic plaques Restriction of mouth opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseudomembranes [†] Ulcers [†]		Gingivitis, mucositis Erythema Pain Food sensitivities
Eyes		Dry, gritty, or painful eyes [†] Cicatricial conjunctivitis Keratoconjunctivitis sicca [†] Confluent areas of punctate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis	
Genitalia	Lichen planus-like vaginal scarring or stenosis	Erosions [†] Fissures [†] Ulcers [†]		
GI tract	Esophageal web Strictures or stenosis in the upper to mid-third of the esophagus [†]		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea Weight loss
Liver				Total bilirubin, alkaline phosphatase $>2-3 \times$ upper limit of normal [†] ALT or AST $>2-3 \times$ upper limit of normal [†]
Lung	BOS diagnosed with lung biopsy [‡]	BOS diagnosed with PFTs and radiology [†]		
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to sclerosis	Myositis/polymyositis [†]	Edema Muscle cramps Arthralgia or arthritis	

Table continues on next page

Table 15-5 Signs and symptoms of chronic GVHD (*continued*)

Body site	Diagnostic of chronic GVHD	Distinctive in chronic GVHD but not diagnostic	Other features*	Common to both acute and chronic GVHD
Hematopoietic and immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hypergamma-globulinemia Autoantibodies (AIHA and ITP)	
Other			Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis Cardiac conduction abnormality Cardiomyopathy	

Adapted from Filipovich AH, Weisdorf D, Pavletic S, et al. *Biol Blood Marrow Transplant*. 2005;11:945–956.

*Can be acknowledged as part of the chronic GVHD symptomatology if the diagnosis is confirmed.

†In all cases, infection, drug effects, malignancy, or other causes must be excluded.

‡Diagnosis of chronic GVHD requires biopsy or radiology confirmation (or Schirmer test for eyes).

recommended for monitoring for late effects and preventive health screening, especially for the problems discussed in this chapter. Many of the late complications seen after HCT are especially profound in younger patients.

Endocrine adverse effects

Endocrine sequelae of myeloablative transplantation may be underappreciated. Children should be followed to ensure that adequate growth is obtained through adolescence. After conditioning with CyTBI, 20% to 70% of children develop growth hormone deficiency. Some children have benefited from growth hormone therapy. In addition, many patients have thyroid dysfunction, often compounded by the effects of therapy before transplantation.

Gonadal tissue damage is common and may result in delayed or absent development of secondary sexual characteristics with the need for sex hormone replacement. The risk for gonadal damage appears to depend on multiple factors, including age, sex, type of transplantation, previous therapy, and conditioning regimen. For many young adults, there is a high risk of infertility after HCT and counseling for sperm or egg banking should be discussed with young patients before HCT. One study of 39 male patients evaluated after HCT demonstrated spermatogenesis in only 28% of the patients. Factors associated with sperm production were age of >25 years at transplantation, longer interval from transplantation, and no chronic

GVHD. Unfortunately, although sperm banking is readily available, only fertilized eggs can be reliably preserved. Research continues on cryopreservation of unfertilized eggs or ovaries. For many patients, the course of their disease does not allow for preservation of gametes; however, counseling with fertility specialists after the procedure, in the future, may allow new options.

Musculoskeletal complications

Patients receiving high-dose corticosteroids for their underlying disease or for GVHD have an increased risk of avascular necrosis of the bone, loss of bone density, and myopathies. Avascular necrosis of the bone can cause progressive collapse of the femoral head, humeral head, and other bones and typically occurs in adolescent and young adult patients treated with corticosteroids. Avascular necrosis is a major cause of morbidity in this age group and frequently leads to intractable pain and loss of mobility, requiring joint replacement at a young age. Loss of range of motion of joints may be seen in patients with a history of chronic GVHD even if the disease is well controlled. Osteoporosis resulting from steroid use and therapy-induced menopause is common. All patients should obtain bone densitometry at 1 year after transplantation and as needed afterwards. Vitamin D deficiency is common, and attention to supplementation of vitamin D may help limit loss of bone density.

Psychosocial considerations

Significant CNS toxicity has been seen after HCT, especially in younger patients receiving intensive intrathecal chemotherapy or cranial radiotherapy before transplantation. Previous evaluations involving quality-of-life assessments completed by parents appear to underestimate the child's quality of life and functioning. Newer methods of neuropsychiatric testing have begun to reveal subtle problems that greatly affect school performance. Identification of these deficiencies and adaptive measures help to improve school functioning. Use of neuropsychiatric testing should be considered on a regular basis for children, as well as younger adults who are finding tasks at home and work more difficult after transplantation. For patients who receive transplantation as adults, changes in executive function, attention, and memory have been reported and may affect the ability to return to a particular job or to continue the previous role of the individual in his or her family life.

Secondary malignancies and posttransplantation lymphoproliferative disorders

Survivors of allogeneic HCT are at increased risk for a variety of second malignancies, including a 2- to 3-fold increased risk of solid tumors compared with their age-matched controls. The risk increases over time after transplantation, with the greater risk among younger patients. In a retrospective multicenter study that included approximately 20,000 patients who had received either allogeneic or syngeneic transplants, the cumulative incidence rates for the development of a new solid cancer were 2.2% and 6.7% at 10 and 15 years, respectively. The risk was significantly elevated for cancers of the buccal cavity, liver, brain, bone, and connective tissue, as well as malignant melanoma. Higher doses of TBI were associated with a higher risk of solid cancers. Chronic GVHD and male sex also were associated with increased risk of squamous cell cancers of the buccal cavity and skin. Patients should be instructed to avoid ultraviolet exposures and to use sunscreens and protective clothing. Dermatologic consultation for close monitoring for and management of skin cancers in high-risk patients should be employed.

PTLDs after allogeneic HCT are usually related to EBV reactivation and complicate approximately 2% to 4% of allogeneic HCTs. They occur more commonly with *in vitro* or *in vivo* T cell-depleted grafts, unrelated or mismatched donors, transplants with highly immunosuppressive GVHD prophylaxis or treatment regimens, and with age >50 years. PTLD may be polymorphic, consisting of nonclonal pro-

liferation of B cells, or monomorphic, manifesting as a clonal proliferation of B cells, often large B-cell lymphoma. Treatment consists of reducing and eliminating immunosuppression, monoclonal antibody therapy with rituximab, donor leukocyte infusions and, in the case of aggressive or unresponsive lymphomas, chemotherapy. EBV-specific cytotoxic T cells hold promise to improve outcomes of PTLD but have yet to be proven feasible for routine use.

Unlike allogeneic HCT patients, long-term survivors after autologous HCT are at considerable risk for therapy-related myeloid neoplasms (myelodysplastic syndromes and acute myeloid leukemia). In some series, the cumulative incidence exceeds 10%. The risk is increased with high-dose TBI used for conditioning and older age, is related to the type and intensity of chemotherapy received prior to conditioning and HCT, and is possibly related to the chemotherapy agents used for stem cell mobilization (high-dose etoposide is thought to confer an increased risk). In some cases, cytogenetic abnormalities are detected in the marrow or stem cell product of patients destined to develop therapy-related myeloid neoplasms, further implicating pretransplantation chemotherapy.

Relapse and the graft-versus-malignancy effect

Relapse is the major cause of treatment failure after autologous HCT and is common after allogeneic HCT. In the setting of autologous HCT, intrinsic disease resistance to chemotherapy and/or radiation, involvement of sanctuary sites with reduced chemotherapy exposure, such as the CNS, and the existence of cancer stem cells that may be quiescent and therefore more resistant to the effects of high-dose chemotherapy and radiation may account for relapse. Maintenance therapies after autologous HCT predictably prolong the time to progression at the expense of ongoing treatment and may improve OS in select diseases (eg, lenalidomide in multiple myeloma), but no maintenance therapy to date has been demonstrated to improve the curative potential of autologous HCT.

In contrast to autologous HCT, allogeneic HCT is associated with a GVT effect mediated by alloreactive donor T cells and B cells that provide an inherent immune surveillance mechanism. The importance of GVT initially was studied by comparing relapse rates between syngeneic (identical twin donor) and allogeneic HCT recipients, by considering the relation between GVHD and relapse, and by examining the effect of T cell depletion of the graft on risk of disease recurrence. Patients with AML in first complete remission (CR1) and chronic myelogenous leukemia (CML) in chronic phase had an increased rate of recurrence after syngeneic HCT relative to allogeneic

HCT. Relapse rates after syngeneic HCT for lymphoma or for acute lymphoblastic leukemia (ALL) in CR1 are not increased compared with allogeneic HCT. Definitive evidence for a GVT effect comes from the use of donor lymphocyte infusions (DLIs). DLI confers a direct graft-versus-malignancy effect by infusion of alloreactive donor lymphocytes, typically in the absence of immune suppression to prevent GVHD. Purposes of DLI include conversion of mixed-donor chimerism to full-donor chimerism after HCT as preemptive therapy to prevent relapse or for the treatment of relapse.

The mechanisms of relapse after allogeneic HCT are poorly defined. These malignancies have not only escaped the effects of high-dose alkylating agents and/or TBI but have also evolved mechanisms to overcome immune-mediated GVT effects. Clonal evolution, loss of specific surface antigens, loss of HLA molecules, and development of immune-suppressive mechanisms have all been postulated.

Treatment and prevention of relapse after allogeneic HCT remains a major challenge. Maintenance therapies such as hypomethylating agents and tyrosine kinase inhibitors have been studied, although the benefit is unclear and tolerability of the treatments low after allogeneic HCT. Preemptive or prophylactic DLI has been attempted, but no large prospective trials have been performed. The application of DLI is not without toxicity and can carry a mortality rate of 3% to 10%, with acute GVHD and marrow aplasia being the leading causes of death. The incidence of severe acute and chronic GVHD after DLI is ~50%, with more than half of the patients who develop chronic GVHD having extensive disease. The onset of acute GVHD typically occurs 32 to 42 days after DLI.

Posttransplant cellular therapies as a strategy for relapse prevention continue to be explored. Novel technologies, such as chimeric antigen receptors or antigen-specific cytotoxic T-lymphocytes, are promising technologies that are in early clinical trials.

Hematopoietic cell transplantation for specific diseases

Mastering the details of HCT is a daunting task: there are various stem cell sources (autologous, allogeneic), donor sources (matched related, unrelated, haploidentical, cord blood), and many diseases with different levels of aggressiveness. Things can be simplified, however, into some basic rules of thumb:

1. Prognosis can be estimated based on disease risk (low, intermediate, high) and stage (low risk for disease in remission vs high risk for relapsed or refrac-

tory disease). Thus, patients with low-risk/low-stage disease have a low overall risk (OS >60%), patients with low-risk/high-stage or intermediate-risk/low-stage disease have an intermediate overall risk (OS approximately 40%), patients with intermediate-risk/high-stage or high-risk/low-stage disease have a high overall risk (OS 25%), and patients with high-risk/high-stage disease have a very high overall risk (OS <10%). The last group may not be good candidates for standard therapies, including HCT, and would instead be best treated in a clinical trial if available.

2. In diseases amenable to an RIC allogeneic HCT, typically those with significant graft-versus-malignancy effect and/or high treatment-related mortality with myeloablative conditioning, survival after “full” myeloablative and RIC conditioning HCT is similar. Relapse is more common after RIC allogeneic HCT, but this is offset by low treatment-related mortality.
3. Different allogeneic hematopoietic cell sources generally yield similar survival outcomes. Thus, survival after matched related, matched unrelated, cord blood, and haploidentical transplants is similar. The differences in these approaches are from the causes of failure (eg, relapse in MRD, GVHD in MUD, infections in cord blood and haploidentical donor transplants).
4. For some diseases, high-dose chemotherapy with autologous HCT is preferred over allogeneic HCT, as autologous HCT can have high cure rates while sparing patients the treatment-related morbidity and mortality associated with allogeneic HCT.

Acute myeloid leukemia

The success of chemotherapy alone in curing AML is largely dictated by leukemia genetics (cytogenetic and molecular abnormalities) and patient age. Chemotherapy alone has high cure rates for the favorable-risk AML: acute pro-myelocytic leukemia and core-binding factor AML [t(8;21), inv16, t(16;16)]. Allogeneic HCT improves survival for patients with poor-risk cytogenetics in CR1 and potentially intermediate-risk AML patients. A meta-analysis of 24 trials comprising 6,007 patients suggested a survival benefit of allogeneic HCT compared with contemporaneous chemotherapy in both poor-risk and intermediate-risk AML. Although intensification of anthracycline during induction improves outcomes for younger AML patients, it has no benefit for patients with poor-risk cytogenetics or those over the age of 65. Ongoing advances in initial therapy for AML such as daunorubicin intensification, liposomal formulation of daunorubicin and cytarabine, the addition of cladribine, and the incorporation of targeted agents into

upfront therapy may change the role of allogeneic HCT for AML in CR1.

Mutations in specific genes can affect prognosis within a defined cytogenetic group. In AML with normal karyotype, a historically intermediate-risk group, mutations in specific genes such as nucleophosmin 1 (*NPM1*), fms-like tyrosine kinase 3 (*FLT-3*), and *CEBPA* significantly alter prognosis. Normal karyotype AML with biallelic loss of *CEBPA* or *NPM1* mutation and no *FLT3-ITD* mutation behave like favorable-risk cases, but patients with a *FLT3-ITD* mutation have very high relapse rates with chemotherapy alone and have relatively good outcomes with allogeneic HCT in CR1. Although considered favorable-risk, *NPM1*-mutant AML may derive a relapse-free (but not OS benefit) from MRD allogeneic HCT over chemotherapy alone. In addition, core binding factor cases with activating mutations in the tyrosine kinase c-Kit have intermediate risk of relapse and death and should be considered for allogeneic HCT in CR1. Older AML patients (often defined as >60 years of age) generally have poor outcomes with chemotherapy alone and thus are candidates for an RIC allogeneic HCT in CR1 if they have limited comorbidities and intermediate- or poor-risk cytogenetic or molecular profiles.

Outcomes of allogeneic HCT in CR1 are predictably better than in CR2 or higher, with active relapse, or with refractory AML. Survival after allogeneic HCT is approximately 40% to 60% in CR1, ~25% to 30% in CR2, and ~10% in refractory AML. These data should not be interpreted to suggest that all patients should receive chemotherapy alone and if not cured expect the same outcome as upfront transplantation by delaying until CR2. About 20% of patients with AML relapsing after CR1 manage to regain remission and survive treatment toxicities to proceed to allogeneic HCT. On the contrary, proceeding to allogeneic HCT with its high treatment-related mortality and morbidity without definitive evidence of superiority over chemotherapy alone does a disservice to favorable-risk patients. Notably, allogeneic HCT for AML refractory to intensive induction or salvage chemotherapy can be curative. A CIBMTR study evaluated outcomes of AML transplanted with persistent disease at transplant and established a predictive score for survival based on CR1 duration <6 months, presence of circulating blasts, donor other than HLA-matched sibling, Karnofsky performance status (KPS) <90, and poor-risk cytogenetics. The authors found a 3-year OS of 19% in the entire AML cohort, but a 3-year OS of 42% in those with a risk score of 0 (favorable findings in all 5 categories of risk).

The optimal myeloablative conditioning regimen for AML has not been determined in a randomized fashion

but current studies support the use of an intravenous busulfan (Bu)-based conditioning (Bu/cyclophosphamide[Cy], Bu/fludarabine[Flu]) rather than TBI-based conditioning. An early, randomized study demonstrated the superiority of TBI/Cy over oral busulfan, a drug with unreliable absorption and significant GI toxicity. Intravenous busulfan has more reliable pharmacokinetics, and 2 studies have reported the superiority of IV Bu-based conditioning over TBI-based regimens. A retrospective CIBMTR retrospective study of 1,230 AML patients in CR1 from 2000 to 2006 comparing IV Bu/Cy conditioning to oral Bu/Cy or TBI/Cy conditioning demonstrated superiority in multivariate analysis of IV, but not oral, busulfan over TBI for leukemia-free survival, OS, relapse, and NRM. A subsequent CIBMTR prospective cohort study enrolling 1,483 patients from 2009 to 2011 compared outcomes of IV Bu-based conditioning to TBI-based conditioning in AML, CML, and MDS and found significantly improved OS and progression-free survival (PFS) with the use of IV Bu-based conditioning relative to TBI-based conditioning with a significant 2-year OS benefit in AML specifically (57% vs 46%, $P=0.003$). As such, IV busulfan-based conditioning has become standard at many transplant centers.

As noted, the encouraging results of RIC regimens has brought this from a treatment for older patients and those with comorbidities into wider use. Indeed, a nationwide randomized phase 3 trial comparing myeloablative vs RIC transplantation in younger patients with MDS and AML (BMT CTN 0901) failed to show a significant OS difference between reduced intensity and myeloablative conditioning, with significantly more relapse with reduced-intensity conditioning and significantly higher treatment-related death with myeloablative conditioning. In cases that have a high likelihood of relapse, however, conventional wisdom suggests as potent a preparative regimen be offered as clinically feasible.

Although a matched sibling donor is still a preferred hematopoietic cell source, principally due to reducing time from CR to transplant and a potentially better match of minor histocompatibility antigens, equivalent outcomes are observed using a fully allele-level HLA-matched unrelated donor. Moreover, it appears that UCB unit and haploidentical donor transplants result in similar outcomes as MRD or MUD transplants. Because most UCB units have slow engraftment in adults due to the relatively small stem cell dose, many centers now use 2 cord units. A major advantage to a UCB is reducing the time from CR to allogeneic HCT. Whereas a typical unrelated donor search may take 2 to 3 months, cord blood units generally are available within a week. In addition, allogeneic HCT using related haploidentical donors is becoming standard

at most centers. The use of posttransplant high-dose cyclophosphamide has made the technique accessible to all transplant centers. Bashey et al performed a retrospective study comparing 53 patients (32% AML) undergoing T cell–replete haploidentical HCTs with posttransplant cyclophosphamide with contemporaneous MRD and MUD HCTs. No significant differences were seen in NRM, relapse, 6-month aGVHD incidence, DFS, or OS between the 3 groups, although significantly less extensive chronic GVHD was seen with haploidentical HCT (38% haploidentical vs 54% MRD vs 54% MUD, $P < 0.05$ for both MRD and MUD). Larger studies are needed to better define the outcomes of haploidentical HCT relative to other stem cell sources because haploidentical donors are often readily available, preventing delays in proceeding to allogeneic HCT, as seen with MUDs. When umbilical cord blood units or haploidentical donors are used, opportunistic infections including rapidly progressive invasive fungal and viral infections (eg, CMV, adenovirus) stemming from delayed immune reconstitution represent a major cause of treatment-related death.

Autologous transplantation for AML has been explored as consolidation for patients in CR1 or CR2. Patients undergoing autologous transplantation in CR1 may have a decreased rate of recurrence compared with those receiving standard chemotherapy but a higher relapse rate than patients undergoing allogeneic HCT. There is controversy whether the higher relapse rates in autologous compared with allogeneic HCT are from AML cells in the stem cell product or the lack of a GVL effect. Either way, the procedure is uncommonly used, given that now almost all patients who need allogeneic HCT have hematopoietic cells available from a matched related, unrelated, or haploidentical donor, or from UCB units.

In AML, multiparameter flow cytometry techniques can measure minimal residual disease as low as 1 AML cell in a background of 10,000 normal cells in some patients who are in morphologic complete remission. More sensitive and broadly applicable genetic techniques are also being developed. Patients with detectable residual disease are at a higher risk of relapse compared with those without detectable residual disease. Thus, it is tempting to use minimal residual disease as a guide to suggest which patients should undergo transplant in remission. Studies have shown that patients with minimal residual disease at the time of transplant do far worse than those without it, with posttransplant relapse rates of 65% vs 18%, respectively, although the utility of minimal residual disease in clinical decision-making is still not clear for AML.

Newly diagnosed acute promyelocytic leukemia (AML FAB M3) has cure rates in excess of 80% with the all-

trans-retinoic acid in combination with chemotherapy or arsenic trioxide. For patients who relapse, salvage therapy is based on prior therapies and duration of remission (< or >6 months). Consolidation with autologous or allogeneic HCT is required to maximize cure rates in these patients. For patients in molecular remission (lacking detectable PML-RAR1 fusion transcript), autologous HCT offers similar DFS to allogeneic HCT but with significantly superior 5- or 7-year OS of 60% to 75% for autologous HCT vs 50% to 52% with allogeneic HCT. For those patients with detectable disease after salvage chemotherapy or relapsing after autologous HCT, allogeneic HCT offers the best opportunity for long-term survival.

Acute lymphoblastic leukemia

The role of allogeneic HCT in ALL differs greatly between the pediatric and adult population. The prognosis of pediatric ALL is excellent with chemotherapy alone resulting in 5-year OS in excess of 80%. Allogeneic HCT in first remission is thus limited to the very high-risk pediatric ALL populations including some children with t(9;22), hypodiploid karyotype, *MLL* rearrangement [eg, t(4;11)], a slow response to therapy, including persistent minimal residual disease, and primary refractory disease. In a retrospective study, children with t(9;22) translocation achieved a 65% long-term event-free survival (EFS) after HCT from an HLA-identical sibling compared with an approximately 25% EFS for patients treated with standard chemotherapy regimens. Several reports of infants with *MLL* rearrangements treated with allogeneic HCT in first remission have documented EFS ranging between 64% and 76%. This compares favorably to an EFS of approximately 33% attained with the most aggressive chemotherapy regimens in this setting.

The superiority of chemotherapy or allogeneic HCT as consolidation for Ph⁻ ALL in CR1 has never been studied in a randomized fashion, rather donor vs no donor comparisons have traditionally been performed. In adults, the role of allogeneic HCT in CR1 is evolving. Some providers have reserved allogeneic HCT for those with high-risk ALL. High risk often is defined by: positive minimal residual disease at end of induction; poor-risk cytogenetics, such as t(9;22), t(4;11), t(1;19), or complex karyotype (>4 –5 abnormalities); WBC $>30,000/\text{mL}$ with the B-cell phenotype; WBC $>50,000/\text{mL}$ with the T cell phenotype; requiring >4 weeks to achieve complete remission; or age >30 to 35 years. Approximately 50% of patients who receive transplantation in first remission become long-term survivors. For patients with standard-risk ALL, several studies have suggested that allogeneic transplant may yield superior results compared with chemotherapy

or autologous transplantation. The largest prospective trial to date addressing this question is the MRC UKALLXII-ECOG2993 trial, which accrued nearly 2,000 newly diagnosed ALL patients from 1993 to 2006. Of the 1,031 Philadelphia chromosome-negative patients in CR with frontline therapy, the 5-year OS among patients who had a donor undergoing allogeneic HCT vs no donor receiving either autologous HCT or chemotherapy was 53% vs 45%, respectively. A meta-analysis of 2,962 Ph⁻ ALL patients from 13 studies comparing chemotherapy with or without autologous HCT to allogeneic HCT showed superior OS in patients under the age of 35 with a matched-sibling donor compared with the no-donor group, due to increased NRM in older patients undergoing allogeneic HCT. Autologous HCT appeared inferior to chemotherapy alone, although this result is complicated by the fact that a large percentage of patients randomized to autologous HCT did not undergo the procedure. Notably, there was no difference in OS when analyzing studies that did not include autologous HCT as part of the comparator with allogeneic HCT. In addition, improved survival outcomes are being observed with the application of intensive adult or pediatric-inspired chemotherapy regimens to younger adult Ph-negative ALL patients, the very group that had a survival benefit in the meta-analysis.

The prognosis of patients with relapsed childhood ALL depends on the site and timing of relapse. Among patients with early marrow relapse (during chemotherapy or within 6 months of stopping maintenance chemotherapy), only 10% achieve long-term EFS with standard chemotherapy. A retrospective review found that children with relapsed ALL had better EFS with allogeneic HCT than with chemotherapy alone for early relapse and that, in this population, a TBI-based regimen was superior. The role of HCT in late ALL relapses in children is debatable due to relatively good results with standard chemotherapy alone.

Despite optimal multiagent chemotherapy for adult ALL, 10% of patients fail to achieve remission after induction and 40% to 60% of patients relapse, historically with very poor long-term survival outcomes of 5% to 10% due in part to a lack of effective salvage options. However, there has recently been a revolution in the treatment of relapsed and refractory ALL with the advent of effective novel therapies allowing more relapsed and refractory patients to proceed to allogeneic HCT beyond CR1. In addition to repeating initial induction in patients with late relapse, several novel treatment options for relapsed/refractory ALL are FDA approved: (1) the bifunctional T cell engager blinatumomab contains variable regions of anti-CD3 and anti-CD19 monoclonal antibodies joined by a

peptide linker and has a CR/CRi rate of 44% in relapsed/refractory B-lineage ALL phase 3 study; (2) inotuzumab ozogamicin is an antibody-drug conjugate linking calicheamicin to an anti-CD22 monoclonal antibody with a CR/CRi rate in relapsed/refractory B-ALL of 81% in a phase 3 study; (3) liposomal vincristine had a CR/CRi rate of 20% as monotherapy in a nonrandomized phase 2; (4) nelarabine yielded a CR rate of 36% in relapsed/refractory T-ALL/LBL; (5) clofarabine-based regimens yield CR/CRp rates of 40% to 60% in small studies; and (6) autologous T cells expressing chimeric-antigen receptors targeting CD19 have shown CR rates of about 90%. All of these therapies may act as a bridge to myeloablative allogeneic HCT, which can yield long-term survival in about 20% to 30% of relapsed/refractory patients. Similar to AML, for those with primary refractory ALL or persistent disease in relapse, myeloablative allogeneic HCT can lead to long-term survival, with multiple relapses, CMV⁺ donor, bone marrow blast percentage >25%, and older age being risk factors for inferior survival.

The role of transplantation in Ph⁺/BCR-ABL1⁺ ALL deserves a special note. Philadelphia-chromosome positivity leading to the BCR-ABL1 fusion protein has long been considered a "high-risk" feature requiring allogeneic HCT, as few patients were cured with chemotherapy alone. The addition of the BCR-ABL1 targeted tyrosine kinase inhibitor (TKI) imatinib to chemotherapy failed to significantly improve survival in adults with a 5-year survival of approximately 20%. The application of second-generation TKIs against BCR-ABL1 (dasatinib, nilotinib), especially when combined with intensive chemotherapy regimens, is changing outcomes, although studies continue to support allogeneic HCT as a preferred treatment. Because long-term follow-up data with second-generation TKIs are not yet available, the conservative approach is to consider patients with Ph⁺ ALL for allogeneic HCT in CR1.

Relapse after allogeneic HCT for ALL is typically incurable. Donor leukocyte infusion has very low response rates in ALL, and its use is controversial. Second allogeneic HCT can be successful in only a small percentage of cases due to high relapse rates (40% to 50%) and NRM. These patients are best treated on a clinical trial or with disease-directed therapies including chemotherapy, blinatumomab or CD19-targeted chimeric antigen receptor (CAR) T cells.

In both pediatric and adult ALL, the detection of minimal residual disease by flow cytometry or by PCR of clonal T cell receptor (TCR) or IgH gene rearrangements is highly predictive of subsequent relapse. Accumulating data suggest that minimal residual disease is the strongest predictor of relapse even in ALL with high-risk features.

An analysis of the GRAALL2003/2005 studies demonstrated that allogeneic HCT in CR1 only benefited high-risk Ph-negative ALL patients with positive minimal residual disease ($\geq 10^{-3}$) at the end of induction. In Ph+ ALL, similar results are emerging as the addition of the second-generation TKIs dasatinib and nilotinib to chemotherapy dramatically improves survival compared to similar combinations with imatinib. As such, allogeneic HCT should be strongly considered in CR1 for all patients with high levels of minimal residual disease (generally $>10^{-3}$ or 10^{-4} , depending on the study). It also is clear that patients with detectable residual disease at the time of transplant or after transplant have inferior outcomes due to high relapse rates compared with patients free of detectable disease, although these patients typically do very well without allogeneic HCT.

Engineering of autologous T cells to express CARs targeting CD19 on the surface of B-lineage lymphoblasts is a novel and powerful strategy currently in clinical trials (see above). Patient T cells are manipulated in vitro to express a chimeric T cell receptor containing an extracellular single-chain variable fragment targeting CD19, a transmembrane domain, an intracellular costimulatory domain(s), and a signaling domain (eg, CD3). The engineered cells are then reinfused into the patient where the cells expand and kill cells expressing CD19, including normal B lymphocytes. Complete response rates in relapsed/refractory ALL are $>80\%$ with a potentially fatal cytokine release syndrome, neurotoxicity, and persistent B-cell aplasia being concerning side effects.

Chronic myelogenous leukemia

CML is driven by the BCR-ABL1 kinase fusion protein generated by the t(9;22) translocation/Philadelphia chromosome. Before the development of TKIs targeting BCR-ABL1, allogeneic HCT was a standard therapy for CML. Allogeneic HCT outcomes differ by CML stage, with chronic-phase CML showing 10-year OS rates of 70% to 80%, accelerated-phase 30% to 40%, and blast crisis $\sim 10\%$. Pretransplant variables define a prognostic scoring system for transplantation in CML. The system devised by Gratwohl using HLA match, stage, age, sex of donor and recipient, and time from diagnosis to transplant is effective in defining posttransplant outcomes following a myeloablative transplant, with EBMT data showing 70% survival for the best score and 20% survival for the worst score.

TKIs targeting BCR-ABL1 (imatinib, dasatinib, nilotinib, bosutinib, ponatinib) have revolutionized treatment of chronic phase CML and imatinib was the first great success of targeted chemotherapy. Primary therapy for chronic phase CML is highly effective and induces complete cytoge-

netic remission (CCyR) in $\sim 80\%$ of cases, with superior response rates but not improved OS for second-generation TKIs (dasatinib, nilotinib) compared with imatinib. Survival at 7+ years is nearly 90%. Approximately 20% to 30% of cases fail primary TKI therapy, however—from intolerance, relapse/resistance, or progression to accelerated phase or blast crisis. For patients receiving secondary therapy for resistant disease, approximately 50% achieve a CCyR. The survival for these patients is $\sim 80\%$ at 3 years. Those who do not achieve and maintain a CCyR often relapse with resistance mutations ABL1 in the BCR-ABL1 oncprotein. Patients with accelerated phase or blast crisis can achieve a CCyR with TKI therapy, but this does not appear to be associated with long-term PFS in the majority of patients. The third generation TKI ponatinib is active against the CML with BCR-ABL1 T315I mutation, with a CCyR in 66% of chronic phase CML patients with the T315I mutation. Omecetaxine is active against CML resistant to TKIs, but the duration of response is typically short.

So, which CML patients should be considered for allogeneic HCT? For chronic phase patients, the initial therapy should be a first- or second-generation TKI. Both the National Comprehensive Cancer Network and the European Leukemia Network have similar guidelines for monitoring response. Roughly 20% of cases become resistant to primary therapy. For those cases that become resistant to imatinib, roughly 40% achieve a CCyR with a second-generation TKI, and some of these cases eventually relapse. Transplantation for chronic-phase CML patients can be considered in the rare cases of intolerance or resistance to all TKIs. Patients relapsing with a T315I mutation should be considered for allogeneic HCT given limited duration of response with ponatinib and lack of effective treatment options after failure of ponatinib. For patients with accelerated phase disease, allogeneic HCT should be considered for poor responders to TKI therapy. All CML patients with myeloid or lymphoid blast crisis should proceed to allogeneic HCT if possible, ideally after successful treatment to CR with a TKI, with or without induction chemotherapy appropriate for myeloid or lymphoid blast crisis.

In the pre-TKI era, several studies showed that prior therapy with busulfan or interferon before transplant was associated with poorer outcomes. This does not appear to be the case with TKIs. Several studies on the effect of prior imatinib and transplant outcomes have failed to show a deleterious effect of pre-transplant imatinib. In addition, there is no evidence that TKI-resistant patients with ABL1 mutations have a poorer outcome following transplantation.

Lastly, CML was one of the earliest malignancies for which a molecular test (quantitative RT-PCR of BCR-ABL1 mRNA) was used to predict subsequent relapse

following transplantation. For patients with detectable molecular, cytogenetic, or morphologic disease, or relapse after allogeneic HCT, treatment generally consists of withdrawal of immunosuppression and a TKI, omacetaxine, and/or donor leukocyte infusion. Given the strong GVL effect in CML, these strategies can be very effective although they run a high risk of stimulating GVHD. Several studies have used TKI therapy to treat “molecular relapse” posttransplant, with remarkable effect. In addition, published and ongoing studies have used TKI prophylactically posttransplant in those cases at very high risk of relapse, such as accelerated and blast phase CML and Ph⁺ ALL.

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)

Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia in North America and Europe. Although this disease usually follows an indolent course, it is typically incurable with standard therapy. Allogeneic HCT has high cure rates with low NRM with the use of reduced-intensity conditioning.

Although newer therapies such as ibrutinib, idelalisib, obinutuzumab, venetoclax, and bendamustine extend treatment options for relapsed/refractory CLL, allogeneic HCT remains appropriate for poor-risk or advanced CLL, broadly defined as cases with primary resistance to purine analogue-containing therapy or relapse within 24 months of initial therapy. Given the aggressive nature of the disease and intrinsic chemotherapy resistance leading to short remission durations in responders, CLL with deletion of 17p14 (with associated p53 loss) and cases with Richter’s transformation of CLL to diffuse large B-cell lymphoma (DLBCL) or HL ideally should undergo allogeneic HCT in first remission with curative intent.

The rise of reduced-intensity conditioning has greatly broadened the use of allogeneic transplantation in CLL, largely supplanting myeloablative approaches, which had a very high NRM (>50%), likely because of the cumulative effects of chemotherapy as well as the older age of the CLL population. A number of studies of reduced-intensity conditioning allogeneic HCT for advanced CLL have been reported and show similar outcomes. The preparative regimens differ, but generally they are based on fludarabine-containing regimens, some with low-dose TBI. In general, 5-year data suggest NRM of ~20%, PFS of ~40%, and OS of 40% to 60%. Of note, 2 studies have shown that, unlike chemotherapy, patients with the p53 mutations or ZAP70 expression fare no differently than other risk groups following reduced-intensity conditioning allogeneic HCT. Not surprisingly, patients who are transplanted with chemosensitive disease and those with low-bulk disease or in

remission do considerably better after transplantation. Indeed, the Seattle group reports that >80% of cases transplanted in CR have remained in CR after 5 years.

Although high-dose chemotherapy with autologous HCT confers high response rates in CLL and reported remission durations lasting up to 5 to 6 years, it is not a recommended modality for CLL. A randomized study from the European intergroup compared autografting with observation in responding patients after first- or second-line therapy. Autologous transplant was associated with reduced relapse rates compared with observation (54% vs 76%, respectively), but OS at 5 years was nearly identical. Moreover, autologous HCT has been associated with posttransplantation MDS/AML with an incidence as high as 12%.

As in ALL, CAR-expressing T cells targeting CD19 have also been used in CLL with success, although with much lower response rates than those seen in ALL. A phase 2 study in relapsed/refractory CLL has yielded an overall response rate of 35%, with 22% of patients achieving a CR.

Myelodysplastic syndromes

Allogeneic HCT is the only curative therapy for MDS. Through the evolution of transplantation regimens, the following has been observed:

1. results are better for early- rather than late-stage disease,
2. outcomes are worse with poor-risk cytogenetics or if the patient has a therapy-related myeloid neoplasm that arises subsequent to prior chemotherapy or radiation therapy,
3. matched-related and unrelated donor HCT yield similar results, and
4. fully myeloablative and reduced-intensity conditioning offer similar survival outcomes, with reduced-intensity conditioning being more effective in patients with low aberrant myeloblast counts.

Like many diseases, outcomes of allogeneic HCT are better in cases of early disease. Thus, cases with refractory anemia have a DFS exceeding 50% (indeed, this may exceed 70% for International Prognostic Symptom Score [IPSS] 0, and 60% for IPSS 0.5 to 1). In contrast, patients with advanced MDS or secondary MDS have an OS closer to 25%. As in AML, cytogenetic risk groups largely map to outcome; again, principally dictated by relapse rates after allogeneic HCT.

The optimal timing of allogeneic HCT for MDS depends on the stage of disease as well as response to supportive therapy and hypomethylating agents. A Markov model

examined 3 approaches to treatment: transplantation right away, transplantation at leukemic progression, and transplantation at a fixed time point (eg, 1 year after diagnosis). Under this model, the transplant-first option was associated with a longer life expectancy in IPSS INT-2 and high-risk disease but delaying allogeneic HCT was the optimal strategy for low and intermediate-1 (INT-1)-risk disease. A subsequent study of nearly 400 patients with myeloablative and RIC transplants showed that increasing age and a time from diagnosis of >12 months were associated with an inferior result. The newer revised IPSS (R-IPSS) better defines prognosis in the low and INT-1 risk groups and adds a very-high-risk group for whom median survival is 9.6 months in the absence of therapy. A decision analysis study suggests that R-IPSS intermediate-, high-, and very-high-risk disease benefits from early allogeneic HCT, whereas very-low- and low-risk disease should delay allogeneic HCT. Based on currently available data, allogeneic HCT is indicated for all transplant-eligible patients with IPSS INT-2- or high-risk or R-IPSS intermediate-, high-, or very-high-risk disease at diagnosis; those progressing to advanced MDS, and those patients with lower-risk disease but failing supportive care with hematopoietic growth factors, immune-suppressive therapy when indicated, and/or hypomethylating agents.

In lower-risk MDS, the risk of delaying transplant is progression to more advanced MDS or AML leading to higher relapse rates after transplantation, the development of complications related to cytopenias, transfusions, or progression to AML that may delay or preclude transplantation; and the need for induction therapy to eliminate blasts prior to transplantation. HLA typing should be initiated after the diagnosis of advanced MDS in transplant-eligible patients and considered in lower-risk patients. If an MRD is not available, an unrelated donor search should be started with consideration of hypomethylating therapy as a bridge to transplant.

Follicular lymphoma (FL)

Follicular lymphoma (FL) typically runs an indolent course but is incurable with conventional chemotherapy. Frontline therapy can lead to prolonged remissions and HCT (either autologous or allogeneic) is reserved for salvage therapy. In the pre-rituximab area, the results of 3 large randomized trials from Europe suggested improved DFS but no benefit in OS for early remission patients randomized to autologous HCT compared with conventional chemotherapy.

For patients with relapsed disease, only 1 prospective randomized trial from Europe, known as the CUP (Conventional Chemotherapy, Unpurged Autograft, Purged

Autograft) trial, has been conducted but closed early due to slow accrual. A total of 89 patients with relapsed disease were randomized to either 3 cycles of salvage chemotherapy vs high-dose therapy with autologous HCT using in vitro purged or unpurged autograft. After a median follow-up of 69 months, the hazard ratio for PFS was significantly better for both autologous HCT arms compared with the salvage chemotherapy arms, and there was a trend for a superior OS favoring the high-dose therapy arms. No difference was seen in outcomes between purged and unpurged autografts. Nonrandomized retrospective studies, including studies from the German Low Grade Lymphoma Study Group and the National LymphoCare Study/CIBMTR, have suggested a survival benefit for autologous transplantation in patients with progression of follicular lymphoma within 2 years of frontline therapy.

There appears to be a strong GVL effect in FL, and thus myeloablative and, more recently, RIC approaches have been used in relapsed disease. Several nonrandomized studies have shown lower relapse rates after allografting compared with autologous HCT, but this gain was offset by the considerably higher NRM with the ablative procedure. RIC allogeneic transplants have been used in FL, including cases failing an autologous transplant. Two prospective studies have used fludarabine-based RIC conditioning regimens. An MD Anderson trial reported 6-year PFS and OS rates of 83% and 85%, respectively, with an NRM of 15%. The Cancer and Leukemia Group B (CALGB) trial reported 2-year PFS and OS rates of 71% and 76%, respectively, with an NRM of only 7%. Patients with chemotherapy-sensitive disease before transplantation fared better than patients with chemotherapy-resistant disease.

Given encouraging results for both autologous and allogeneic HCT in relapsed follicular lymphoma, which strategy best serves patients? The EBMT performed a retrospective analysis comparing autologous ($n=726$) to RIC allogeneic HCT ($n=149$) as first-transplant strategy in relapsed follicular lymphoma. Relative to autologous HCT, RIC allogeneic HCT yielded significantly reduced relapse rates and longer PFS at the expense of increased NRM leading to equivalent 5-year OS (72% autoHCT vs 69% alloHCT, $P=NS$). Patients undergoing RIC allogeneic HCT had increased early death compared with autologous HCT and 2-year cumulative incidence of acute GVHD and chronic GVHD of 47% and 52%. For 292 patients relapsing after autologous HCT, 56 underwent RIC allogeneic HCT with 3-year PFS and OS of 39% and 50%, respectively.

The incidence of transformation from FL to diffuse large B-cell lymphoma is ~3% per year, with several stud-

ies reporting a risk of 30% by 10 years of follow-up. Chemotherapy alone is unlikely to be curative. Autologous transplant has been associated with a 5-year OS of ~40% to 60%, with EFS or PFS ranging from 25% to 50%. Aberrant allogeneic transplants have not done better because of high NRM. RIC approaches are being studied, with PFS and OS ranging from 20% to >60%, likely owing to the differences in study populations (particularly chemotherapy responsiveness).

Diffuse large B-cell lymphoma

The majority of patients with aggressive and very aggressive B-cell NHLs can be cured with frontline combination immunochemotherapy, with or without consolidative radiotherapy. For patients relapsing after initial chemotherapy, autologous or allogeneic HCT can be curative, but securing a remission durable enough to proceed to HCT can be difficult, leading to numerous studies of autologous HCT in the frontline setting. In the pre-rituximab era, the GELA LNH87-2 study randomized DLBCL patients in CR1 to consolidation with standard dose chemotherapy or high-dose chemotherapy with autologous HCT. For patients with an age-adjusted International Prognostic Index (aaIPI) of high/intermediate or high-risk disease, 8-year DFS and OS were significantly improved with upfront autologous HCT. Several subsequent randomized studies in the rituximab era have failed to show a consistent PFS or OS benefit to upfront autologous HCT when compared with standard chemoimmunotherapy; except, perhaps, SWOG 9704 that in a retrospective cohort analysis demonstrated superior 2-year PFS and OS with autologous HCT in IPI high-risk DLBCL. As it stands, upfront autologous HCT for DLBCL in CR1 should be reserved for rare high-risk patients, ideally within the context of a clinical trial.

For patients failing to achieve CR with initial therapy or for patients with relapsed disease, standard-dose salvage therapy with chemotherapy alone is not curative. High-dose chemotherapy with autologous HCT offers curative potential and is the treatment of choice for patients with relapsed, chemotherapy-sensitive DLBCL. Autologous HCT is the standard of care for most patients with chemotherapy-sensitive relapsed or refractory DLBCL. The international, multicenter, prospective PARMA trial established the role of autologous HCT for patients with relapsed, chemotherapy-sensitive DLBCL. In this trial, 109 of 215 patients who had relapsed DLBCL and responded to platinum-based salvage chemotherapy were assigned randomly to 4 more courses of conventional chemotherapy or autologous HCT. The 5-year EFS and OS were 46% and 53%, respectively, for the transplantation arm

and 12% and 32%, respectively, for the chemotherapy arm. Patients with relapsed DLBCL who were chemotherapy-sensitive unequivocally fared better compared with patients with chemotherapy-resistant disease.

Patients with DLBCL who demonstrate primary refractory disease or relapsed disease that is not responsive to salvage chemotherapy have poor outcomes even after high-dose therapy with autologous HCT. Autologous HCT is indicated for patients who respond to salvage therapy after demonstrating resistant disease to frontline therapy. Salvage chemotherapy regimens and their results are discussed in the relevant section of this book.

The introduction of rituximab has improved the prognosis of DLBCL, and rituximab has a growing role in the peritransplantation management of DLBCL. Rituximab is part of frontline therapy and is typically added to salvage regimens (eg, R-ICE, R-DHAP) for added cytoreduction and an *in vivo* purging effect that may reduce the incidence of tumor contamination in the autograft. The international phase 3 CORAL study randomized refractory or first-relapse DLBCL patients to salvage with R-ICE or R-DHAP followed by high-dose therapy with BEAM (BCNU, etoposide, cytarabine, melphalan) with autologous HCT with a second randomization to rituximab maintenance or no maintenance after transplant. Response rates were nearly identical for R-ICE and R-DHAP, with about 50% of patients in each arm proceeding to autologous HCT. PFS was similar if patients went to transplant in CR or PR. Notably, rituximab maintenance had no effect on EFS or OS compared with observation alone.

Several prognostic factors are associated with outcome after autologous HCT. The IPI is the validated scoring system designed to predict survival of patients with newly diagnosed aggressive NHLs. The aaIPI at relapse (second-line aaIPI: 1 point each for LDH >upper limit of normal, stage III or IV disease, KPS <80%), however, also has been shown to correlate with prognosis after autologous HCT. In addition, positron emission tomography (PET) scanning has predictive value. Several studies have shown that PET positivity after salvage therapy is associated with an inferior failure-free survival independent of aaIPI. Other poor prognostic features include relapse within 12 months of diagnosis, advanced stage, poor performance status, and failure to achieve CR after transplantation.

Allogeneic HCT is not offered routinely to patients with DLBCL. Exceptions include select young patients with advanced disease, patients who failed to mobilize adequate CD34⁺ hematopoietic cells, or patients who failed a previous autologous HCT. In a review of 101 patients with DLBCL who failed an autologous transplant, 3-year NRM was 28% (higher in myeloablative vs RIC), relapse

was 30%, and OS 52%. Time to relapse of <12 months and chemotherapy-refractory disease at transplant portended a worse outcome.

Peripheral T cell lymphomas (PTCLs)

Peripheral T cell lymphomas (PTCLs) account for 10% of NHLs and are generally aggressive lymphomas. With the exception of ALK+ anaplastic large cell lymphoma, which has 5-year OS rates of 60% to 70% with CHOP or CHOP-like chemotherapy alone, PTCLs tend to have poorer response to and shorter survival after chemotherapy alone compared with DLBCL. The 5-year OS of these PTCLs is approximately 40%. Outcomes with autologous HCT are good but not clearly superior to chemotherapy, especially in patients achieving a CR. For relapsed or refractory disease, autologous HCT yields 5-year survival rates of about 40% in chemosensitive disease, with similar outcomes observed for allogeneic HCT with myeloablative or RIC conditioning.

Mantle cell lymphoma

Mantle cell lymphoma is an uncommon lymphoma (5% to 10% of lymphomas) with a male predominance that generally presents with advanced disease. Traditionally, less aggressive chemotherapy alone (eg, CHOP) does not offer durable disease control in most cases and results in a median OS of approximately 3 years. Most younger, newly diagnosed patients receive aggressive therapy with regimens using rituximab, cyclophosphamide, vincristine, doxorubicin, and prednisone with the addition of high-dose cytarabine-containing courses (eg, CALGB 59909, R-HyperCVAD, Nordic MCL-2) which yield overall response rates of ~90%. High-dose therapy with autologous HCT is typically part of consolidation therapy yielding 5-year PFS of 50% to 70% and 5-year OS of 60% to 70%, with low rates of relapse after 5 years; although it is still not clear if autologous HCT is curative in a portion of patients. Higher proliferation fraction as measured by Ki67 immunohistochemistry is associated with shorter EFS. Rituximab maintenance after frontline autologous HCT for mantle cell lymphoma is currently under study. The phase 3 LyMa study randomized 299 mantle cell lymphoma patients (aged 27 to 65 years) treated with R-DHAP followed by R-BEAM autologous HCT to maintenance rituximab or observation after HCT. Relative to observation, patients treated with rituximab maintenance showed improved 4-year EFS (79% vs 61%, $P=0.001$), PFS (83% vs 64%; $P=0.015$), and OS (89% vs 80%, $P=0.04$), suggesting an important role for rituximab maintenance after upfront autologous HCT, at least in the context of the LyMa regimen.

For patients with relapsed or refractory disease after autologous HCT, allogeneic HCT is indicated as it offers the only chance for cure and long-term survival. Myeloablative allogeneic HCT can induce durable remissions in mantle cell lymphoma, even in heavily pretreated patients, but is associated with significant treatment-related mortality and morbidity. Reduced-intensity conditioning regimens reduce toxicity without significantly sacrificing curative potential, yielding EFS rates ranging from 50% to 85% even in patients who failed a prior autologous HCT.

Classical Hodgkin lymphoma

Frontline therapy for classical HL has high cure rates. For the unfortunate patients with relapsed or refractory disease, high-dose therapy with autologous HCT is the standard of care and confers cure rates of 40% to 60% in patients with relapsed, chemotherapy-sensitive disease and 25% to 40% in patients with chemotherapy-refractory disease. Maintenance therapy after autologous HCT has also been evaluated for classical HL. Brentuximab vedotin (BV) is an antibody-drug conjugate targeting CD30 on the cell surface and is FDA approved for classical Hodgkin lymphoma relapsing after autologous HCT and relapsed/refractory CD30+ anaplastic large cell lymphoma. The AETHERA trial, a randomized, double-blind, placebo-controlled phase 3 study comparing 16 BV treatments after autologous HCT to placebo for classical HL at high risk for relapse or progression, demonstrated superior PFS (HR 0.57, $P=0.001$) with BV but at the expense of increased sensory and motor neuropathy, grade 3 to 4 neutropenia, and a low rate (2 cases) of fatal acute respiratory distress syndrome attributable to BV. Like many other maintenance studies in lymphoma, however, no OS benefit has been observed to date, perhaps in part due to effective salvage with BV. The lack of an OS benefit suggests a limited role, if any, for posttransplant BV given the overtreatment of a large percentage of patients who would never have needed the drug because they were cured by autologous HCT; and yet incur resultant toxicities and the high expense of the drug.

The role of allogeneic HCT in classical HL is less established and generally pursued only in patients who have persistent marrow involvement, refractory disease, or relapsed or progressive disease after an autologous HCT. In general, RIC transplant regimens are preferred to a fully myeloablative regimen, as RIC is associated with fewer regimen-related deaths and better survival in a population that typically has previously undergone a myeloablative autologous HCT. The Gruppo Italiano retrospectively compared nearly 200 classical HL cases following a failed autologous transplant and divided the patients into those

with a donor (sibling, unrelated, or haploidentical) vs those who could not secure a donor, with the intent that those with donor would have an RIC allogeneic HCT. The 2-year PFS and OS were superior in the donor group (39% vs 14% and 66% vs 42%, respectively). The Seattle group compared the outcome for HLA-matched, unrelated matched, and haploidentical donors in RIC allogeneic HCTs and found survival to be similar in all approaches, with OS of ~60%, and PFS of ~40%. Chemosensitivity before RIC allogeneic HCT predicts reduced risk of relapse.

Plasma cell dyscrasias

Multiple myeloma is the most common indication for autologous HCT. Compared with chemotherapy, high-dose therapy with autologous HCT is associated with higher response rates and improved PFS and OS. When autologous HCT is given as part of the planned frontline treatment, ~22% to 44% patients achieve CR, with median time to progression and OS of 18 to 24 months and 4 to 6 years, respectively. High-dose melphalan alone at a dose of 200 mg/m² is the most commonly used preparative regimen for patients with multiple myeloma undergoing autologous HCT. The procedure is well tolerated, with a treatment-related mortality of ~2%.

The advent of the immunomodulators (imids such as thalidomide and lenalidomide) and proteasome inhibitors (such as bortezomib) as part of frontline treatment for myeloma has changed the treatment paradigm. The newer agents result in more patients attaining CR, near CR, and very good partial response with frontline therapy. Current guidelines state that high-dose chemotherapy with autologous stem cell transplantation should be offered as initial consolidation therapy in patients with newly diagnosed myeloma who are <65 years old and have a good performance status. It appears, however, that patients with adverse prognostic features at diagnosis (such as high serum β_2 -microglobulin) or an unfavorable karyotype (such as deletion 13 and deletion 17p), still have poor outcomes even after tandem (double) autologous HCT.

The PFS and OS benefit of upfront autologous HCT relative to chemotherapy alone appears to continue in the era of thalidomide and lenalidomide. Palumbo et al randomized newly diagnosed multiple myeloma patients to 200 mg/m² melphalan with autologous HCT or chemotherapy alone with melphalan, prednisone, and lenalidomide (MPR) followed by a second randomization in each arm to maintenance lenalidomide or no maintenance. Two major results of the study were a significantly improved OS with autologous HCT relative to MPR alone (HR 0.55; $P=0.02$) and improved PFS but not OS with lenalidomide maintenance vs no maintenance.

Because of concerns over the potential toxicities associated with HCT, a strategy of delayed transplantation is undergoing continued study. In a French randomized study, upfront transplantation was compared with transplantation at relapse with stem cells collected at diagnosis. Early transplantation significantly improved PFS, but there was no difference in OS. Early transplantation, however, was associated with a shorter period of chemotherapy and hence improved quality of life. Given the advent of new drugs like lenalidomide, bortezomib, and daratumumab, it is no longer clear that there is a benefit to autologous HCT in first response, or what groups of myeloma patients might benefit. An ongoing randomized phase 3 trial is comparing initial therapy with lenalidomide, bortezomib, and dexamethasone with or without high-dose melphalan with autologous HCT in untreated multiple myeloma to determine if the survival benefit of autologous HCT as first consolidation remains since the advent of imids and proteasome inhibitors.

Currently, the utility of tandem transplantation, either auto-auto or auto-allo, as part of primary treatment remains controversial. In a randomized study from the French group, both response rates and survival favored tandem autologous HCT over single autologous HCT, in particular for patients with significant residual disease (the lack of at least a very good partial response) after their first transplant. Event-free, relapse-free, and overall survival were 10%, 13%, and 21%, in the single-transplant group, compared with 20%, 23%, and 42% in the tandem transplant group. A meta-analysis of 6 randomized trials with ~1,000 patients concluded that tandem autologous HCT confers higher response rates compared with single autologous HCT, but it did not find conclusive evidence for improvement in PFS or OS. A registry analysis from the EBMT, however, demonstrated that when a second transplantation is performed within 3 to 6 months after the first transplantation, survival is improved.

Relapse is the overwhelming cause of autologous HCT failure. Allografting potentially provides a stem cell source free of myeloma cells and a graft-versus-myeloma effect, but myeloablative allogeneic HCT has been associated with unacceptably high treatment-related mortality. Thus, there has been interest in using RIC transplants after an autologous transplant. Numerous studies have compared tandem autologous HCT to autologous HCT, followed by RIC allogeneic HCT, as part of upfront therapy for multiple myeloma with mixed results. Arneson et al conducted a meta-analysis of 6 trials comprising 1,192 subjects undergoing tandem autologous HCT and 630 subjects undergoing autologous HCT followed by RIC allogeneic HCT. Treatment-related mortality was significantly higher in

the allogeneic HCT group without any benefit seen for PFS or OS with the autologous-allogeneic HCT strategy. At this time, single autologous HCT after response to primary therapy remains the standard at most institutions, and other approaches are best performed in the setting of a clinical trial. Allogeneic HCT remains an option for patients relapsing after autologous HCT.

Multiple myeloma was the first disease to demonstrate a clear disease progression benefit with maintenance therapy after autologous HCT. In 2 large randomized trials, post-transplant lenalidomide therapy resulted in significant improvements in DFS. In a randomized placebo-controlled North American trial reported by McCarthy et al, lenalidomide maintenance led to significantly improved time to progression (46 months for lenalidomide vs 27 months for placebo, $P < .001$) and OS (2-sided $P = 0.03$, 3-year OS 88% vs 80%). The IFM2005-02 study randomized myeloma patients to lenalidomide or placebo after autologous HCT and found a similar PFS benefit but no difference in OS with lenalidomide maintenance. In both studies, lenalidomide had more hematologic adverse events, and secondary primary cancers occurred more frequently in lenalidomide maintenance patients compared with placebo.

AL (light chain amyloid) amyloidosis is a clonal plasma cell disorder characterized by tissue deposition of amorphous extracellular material composed in part of immunoglobulin light- or heavy-chain fragments in many vital organs, such as the heart, lung, kidney, liver, and CNS. This infiltrative process ultimately leads to organ failure and death. The prognosis of patients with AL amyloidosis is poor, with median survival of ~1 to 2 years. Although conventional chemotherapy has limited utility in patients with AL amyloidosis, autologous HCT can reverse the disease process for selected patients. Nonimmunoglobulin (non-AL) forms of amyloidosis, however, do not benefit from cytotoxic therapy, including transplantation. Because of the preexisting organ dysfunction in patients with AL amyloidosis, the NRM of autologous HCT is 2 to 5 times (NRM ~5% to 10%) higher compared with that of autologous transplantation for multiple myeloma (NRM ~2%). The causes of NRM include GI bleeding, cardiac arrhythmias, and the development of intractable hypotension and multiorgan failure. Several studies have suggested a 2- to 3-year survival of ~70% after autologous transplant, although patients with multiorgan involvement have a distinctly worse survival. A phase 3 trial in which AL amyloid patients were randomized to receive autologous stem cell transplantation vs oral melphalan and high-dose dexamethasone suggested a benefit of the conventional chemotherapy arm because of the high NRM of 24% in the transplant arm. After a median follow-up of 3 years,

the OS was significantly longer in the conventional-dose group (57 vs 22 months; $P = 0.04$).

POEMS syndrome is a rare condition characterized by polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes, as well as a clonal plasma cell disorder. Investigators from the Mayo Clinic performed transplantation in 16 patients with POEMS syndrome; 15 patients had a severe, rapidly progressive sensorimotor polyneuropathy, and 9 patients were wheelchair dependent. All 14 evaluable patients achieved neurologic improvement or stabilization. Other symptoms also improved substantially. Autologous HCT results should be considered a therapeutic option in these patients.

Aplastic anemia and bone marrow failure syndromes

Therapy for aplastic anemia depends on the severity of the aplasia, the availability of an MRD, and the age of the patient. The standard first-line therapy for younger patients with newly-diagnosed severe aplastic anemia is allogeneic HCT if a matched-related donor is available. If a matched-related donor is not available or a patient is an older adult, immunosuppressive therapy (IST) with horse ATG and cyclosporine (with or without the thrombopoietic receptor agonist eltrombopag) is used for initial therapy with unrelated donor transplant reserved for patients who do not adequately respond to IST. Long-term survival following an MRD transplant exceeds 80%. Inferior survival is associated with older age, use of an unrelated donor, and prior transfusion. The main complication of transplant is related to chronic GVHD, which, unlike in hematologic malignancies, has no benefit in terms of reduced risk of relapse. Thus, bone marrow rather than peripheral blood is the highly preferred source of stem cells to reduce the risk of chronic GVHD. In regard to preparative regimen, most use high-dose cyclophosphamide (50 mg/kg \times 4 doses) with ATG, although regimens incorporating fludarabine with ATG and lower doses of cyclophosphamide are highly efficacious with less toxicity.

For younger patients (often defined as <40 years of age) with newly diagnosed idiopathic severe aplastic anemia and an HLA-identical sibling, many centers recommend immediate transplantation to minimize alloantigen sensitization with transfusions, which historically has resulted in an increased risk of graft rejection and poorer outcomes. Although the use of cyclosporine, as well as leukodepleted blood products, has reduced the problem of rejection, sensitization should be minimized through strict avoidance of transfusions when possible and avoidance of directed family donations of blood products. Indeed, one large study showed the hazard ratio for mortality was 1.7 for pa-

tients who received IST before transplant, compared with those patients who underwent frontline transplantation. Secondary malignancies occur after transplantation for severe aplastic anemia in as many as 10% of cases 15 years from transplant. Risk factors include age >15 years, use of cyclosporine in an IST regimen before transplant, and perhaps radiation therapy as part of the transplant regimen (no longer preferred, as noted).

It is important to assess for Fanconi anemia and dyskeratosis congenita as congenital causes of bone marrow failure in newly diagnosed aplastic anemia patients to help select the appropriate treatment course and avoid the futile administration of immune-suppressive therapy that is standard in idiopathic aplastic anemia. Patients with Fanconi anemia frequently do not have all of the stigmata of the disease, and the diagnosis is overlooked easily. The sensitivity of patients with Fanconi anemia to alkylating agents is well known, and transplantation can be done successfully using only NMA regimens. Recent trials have focused on reducing radiation exposure in addition to reducing doses of alkylating agents in these patients. Patients with Fanconi anemia are at high risk for solid tumors, especially following radiation exposure.

Dyskeratosis congenita is a congenital bone marrow failure syndrome caused by mutations in telomerase or telomerase-associated genes. Most patients develop abnormal skin pigmentation, nail dystrophy, and oral leukoplakia. Bone marrow failure occurs early in life, necessitating allogeneic HCT to prolong life in most patients. RIC/NMA allogeneic HCT minimizing the use of alkylators and radiation in conditioning is preferred, as patients are prone to pulmonary fibrosis, hepatic cirrhosis, and secondary malignancies. As for other inherited disorders, siblings should be screened for the recipient's bone marrow failure syndrome.

Autoimmune diseases

Given its immunosuppressive properties, autologous transplantation has been studied as treatment for life-threatening autoimmune disorders. Autologous HCT has been used in multiple sclerosis, systemic sclerosis, rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, dermatomyositis/polymyositis, Crohn disease, and autoimmune cytopenias. The therapeutic rationale for these transplantations is that high-dose chemotherapy may eradicate or modulate clones of autoreactive T cells. Although the integration of this approach into treatment of each disease depends on the results of ongoing trials, some general observations are now possible. First, allogeneic HCT has considerable treatment-related morbidity

and mortality in this population and is not typically used outside of a clinical trial. Second, the underlying organ dysfunction often progresses acutely during transplantation even if there is stability or improvement later. Third, durability of response and the need for continued therapy after HCT remain to be defined. The waxing and waning course of autoimmune disorders makes it difficult to define end points in these diseases. Results that have been considered encouraging in the transplantation literature have been considered disappointing (both regarding the rates of response and toxicity) in the rheumatology literature. Nonetheless, patients with aggressive autoimmune disorders should consider clinical trials and examine this approach as one of their treatment options.

Hemoglobinopathies

Thalassemia major

The Pesaro, Italy, team has pioneered transplantation for thalassemia and reported high cure rates. Three factors predict adverse transplantation outcomes: hepatomegaly (>2 cm below the costal margin), hepatic fibrosis, and irregular chelation. Quality chelation therapy is defined as deferoxamine therapy initiated <18 months after the first transfusion and given for >5 days each week. Class I patients have none of these factors; class II patients have 1 or 2 factors; and class III patients have all 3 factors. For class I patients <17 years of age, survival, thalassemia-free survival, NRM, and recurrence of thalassemia were 94%, 87%, 6%, and 7%, respectively. The rates of survival, thalassemia-free survival, NRM, and recurrence of thalassemia were 84%, 81%, 15%, and 4%, respectively, for class II patients. Patients with class III disease have more complications and a higher rate of graft rejection. The probability of thalassemia-free survival for young patients who are in class III is 62%, and the risk of dying is 35%. RIC regimens have been investigated in these patients. Class III adults receiving reduced-dose conditioning appear to have a lower rate of rejection. The Pesaro team noted a 24% chance of rejection if the individual has received >100 transfusions, compared with a 53% chance in patients who have received fewer transfusions.

The optimal source of stem cells for patients with hemoglobinopathies is still under investigation. To avoid chronic GVHD, the use of bone marrow rather than PBSCs has been advocated. For those lacking sibling donors, unrelated and cord blood donor transplants have shown promising results in both pediatric and adult patients, provided donor compatibility is stringent. Cord blood transplantation has been used in cases without a matched sibling or unrelated donor. The 2-year probability of survival after

cord blood transplantation for children with thalassemia was 79% in 33 patients who received transplantation. Unfortunately, nearly a quarter rejected the graft.

Patients with thalassemia major frequently develop mixed chimerism following transplantation, which often leads to marked improvement in their transfusion requirements. The patients remain at risk for graft rejection, however, especially those whose percentage of host cells remains >25%.

Sickle cell disease

Allogeneic transplantation is a promising therapy for sickle cell disease. Results from children who have received transplantation from HLA-identical siblings show a >90% survival rate, and 85% are disease free. Moreover, successful allogeneic HCT appears to prevent further sickle cell complications. A study from Belgium demonstrated that patients who received transplantation early in their disease (less than 4 blood transfusions) had a 100% survival rate and 93% DFS rate, compared with an 88% OS rate and 80% DFS rate in patients who received transplantation later in the course of their disease.

Despite these successes, many recommend reserving transplantation for children at high risk from their sickle cell disease because of the toxicities and risks. Frequently, however, children at significant risk are not identified until they have suffered end-organ damage, including stroke or severe lung injury. In addition, the clinical course for a patient may vary over time. Attempts to identify risk factors of severe disease have suggested high WBC count, severe anemia, and early dactylitis as surrogate markers. But the ability to predict the clinical course for each individual remains elusive. In addition, finding suitable, unaffected sibling donors has been difficult. In one study, only 14% of patients with siblings had a suitable HLA-matched donor.

NMA allografting has been studied in adults. A preparative regimen including pretransplant alemtuzumab (an antibody therapy to CD52, which reduces B and T cells), 300 cGy of TBI, and posttransplant sirolimus following HLA-matching sibling CD34+ PBSC infusion has been used to remarkable effect. All 10 patients were alive at 30 months of follow-up, and 9 of 10 patients had stable-donor chimerism. Remarkably, there were no cases of acute or chronic GVHD.

Immune deficiency disorders

Many immune deficiency disorders become evident in infancy secondary to an increased rate of infections or to the presence of opportunistic infections. In such cases, the possibility of HIV infection must be ruled out. For patients suspected of having a primary immune deficiency, definitive diagnosis of the exact molecular defect is impor-

tant to predict the course of the disease and to be able to tailor therapy appropriately. The most common diseases for which allogeneic transplantation is indicated include severe combined immunodeficiency syndrome (SCIDS), adenosine deaminase deficiency, Wiskott-Aldrich syndrome, Nezelof syndrome, Omenn syndrome, MHC antigen deficiency, leukocyte adhesion defect, Chédiak-Higashi syndrome, chronic granulomatous syndrome, and DiGeorge anomaly.

Newborns known to have or to be at high risk for severe SCIDS should be isolated at birth because infection increases the risk for complications of allogeneic HCT. Evaluation of early complete blood counts may suggest a neutrophil (neutrophil adhesion disorder or Kostmann syndrome) or lymphocyte disorder, such as SCIDS. Cord blood, when available, should be studied for lymphocyte numbers and in vitro function. HLA typing should be undertaken as soon as a diagnosis of SCIDS or other combined deficiency potentially correctable by allogeneic HCT is established. Allogeneic HCT approaches are modified based on the exact diagnosis. The need for a preparative regimen and its intensity of conditioning are determined in part by the function of the lymphocytes and NK cells.

Allogeneic HCT is undertaken in these disorders to provide a stable source of immunologically competent cells. The major complications are rejection of the marrow graft and GVHD. Graft rejection occurs when sufficient immune function remains for the recipient to mount a cellular immune response against donor cells. In some forms of SCIDS with absent T cell function, such as X-linked SCIDS, Janus kinase 3 (Jak3) deficiency, and complete recombination activation gene-1 (*RAG-1*) and recombination activation gene-2 (*RAG-2*) deficiencies, the patient is unable to reject the hematopoietic cells. In these patients, simple infusion of hematopoietic cells is usually all that is required, without a preceding preparative regimen. Many of the recipients who received hematopoietic cells without a preparative regimen failed to develop normal B-cell function and required ongoing IgG replacement with intravenous immunoglobulin. This has led many centers to tailor the preparative regimen to include some chemotherapy (most recently fludarabine) to attempt to ensure full immune reconstitution. Patients with adenosine deaminase deficiency, the largest subset of this group, require a preparative regimen despite the absence of detectable T cell function because the donor lymphocytes may rescue the host cell function, thus allowing for ultimate graft rejection. Patients with normal NK cell activity (including some X-linked, Jak3, and RAG defects) also often require preparative regimens, again emphasizing the need to determine the exact defect before initiating therapy.

Results of transplantation are best for children receiving HLA-identical sibling transplants, with survival ranging from 70% to 100%. For patients lacking a sibling donor, results have ranged from 30% to 50%. In the past, many patients lacking a sibling donor have received haploidentical grafts from a parent, although the increasing availability of cord blood stem cells provides another option. Cord blood stem cells are particularly appealing because they can be accessed readily and are not infection carriers, decreasing the risk of CMV disease and EBV lymphoproliferative disorders after transplantation.

Inherited metabolic disorders

A number of inborn errors of metabolism can be corrected with allogeneic HCT. One of the most important steps is the early identification of the disorder before the development of end-organ damage. The role of transplantation varies according to the disorder identified. For instance, certain storage disorders such as Niemann-Pick type IA disease are not treatable by transplantation. Other disorders such as globoid cell leukodystrophy, metachromatic leukodystrophy, adrenoleukodystrophy, mannosidosis, fucosidosis, aspartylglucosaminuria, Hurler, Hunter, Maroteaux-Lamy, and Sly syndromes, and Gaucher disease type III, have been treated successfully with allogeneic HCT. Siblings and parents should be HLA typed as soon as possible. For some of these disorders, transplantation using marrow from a donor heterozygous for the trait does not cure the disease. For those lacking a suitable related donor, the best donor source is unclear. The pace of the disease may make the time required for the typical search for a MUD unrealistic, thus making cord blood cells more attractive in these cases. GVHD in some of these disorders (eg, adrenoleukodystrophy) may accelerate their disease process and increase the risk of rapid decline. The timing of the transplantation may be difficult because not all patients with the same apparent diagnosis have the same course of disease. Thus, in adrenoleukodystrophy, some patients have rapid neurologic decline at an early age, whereas others may not manifest symptoms until later in childhood, adolescence, or adulthood, if at all. In a number of these disorders, HCT halts the disease progression, but the patient may not regain lost milestones or function and may actually show more rapid deterioration.

Hematopoietic cell transplantation for solid tumors

Germ cell cancer

Germ cell cancers are highly curable, even in patients with disseminated disease. Although conventional-dose cisplatin-based chemotherapy cures the majority of pa-

tients, patients presenting with advanced disease have a somewhat higher rate of recurrence. Some patients at first relapse can achieve a durable remission with salvage chemotherapy, but most of the patients who fail salvage chemotherapy or have cisplatin-refractory disease ultimately die of the disease. Approximately 15% to 20% of patients with multiply relapsed or overtly cisplatin-refractory germ cell cancer, however, can be cured with high-dose carboplatin and etoposide followed by autologous HCT. In a large retrospective study, progressive disease before transplantation, primary mediastinal tumor, refractoriness to conventional-dose cisplatin, and human chorionic gonadotropin levels >1,000 IU/L before transplantation predicted transplantation failure. The estimated 2-year survival rates were 51% and 5% for patients with no risk factors and multiple risk factors, respectively.

Transplantation has been investigated as consolidation therapy after initial treatment of patients with advanced disease. In an EBMT prospective study, patients were randomized between 4 cycles of etoposide, ifosfamide, and cisplatin (VIP) vs 3 cycles of VIP plus a single cycle of high-dose therapy followed by autologous HCT. The 3-year EFS for patients who received VIP only was 35% vs 42% for patients randomized to transplantation, with no difference in OS. A US intergroup phase 3 randomized study failed to demonstrate any benefits in high-dose therapy for patients with newly diagnosed intermediate- or poor-risk germ cell cancer.

Pediatric solid tumors

Many pediatric solid tumors demonstrate exquisite chemosensitivity, leading to the exploration of autologous HCT as a method of dose intensification for children presenting with high-risk or recurrent disease.

Neuroblastoma

In 1999, the Children's Cancer Group reported a study of >500 patients with high-risk neuroblastoma (defined as age >1 year, metastatic disease, amplification of *MYCN* oncogene, and histologic findings). All patients were treated with the same initial regimen of chemotherapy, and those with progression of disease were assigned randomly to more chemotherapy or HCT using purged autologous bone marrow. Patients still without disease progression were then randomized to differentiation therapy with 13-*cis*-retinoic acid or no further therapy. The 3-year EFS was superior for the HCT group (34% vs 22%). Among patients assigned to receive *cis*-retinoic acid and HCT, EFS was 55% vs 18% in those assigned to chemotherapy and no *cis*-retinoic acid. More recently, the use of purged mobilized PBSCs has replaced purged bone

marrow at many centers and is associated with decreased HCT-related mortality. Ongoing studies are investigating additional HCT-related strategies to further improve the outcome of high-risk patients, such as the use of sequential autologous transplantations and combination therapies with high-dose radiopharmaceutical agents such as iodine-131 metaiodobenzylguanidine.

Ewing sarcoma

Like neuroblastoma, the Ewing sarcoma and primitive neuroectodermal tumor family includes chemotherapy-sensitive and radiotherapy-sensitive tumors. High-risk features of Ewing sarcoma include a large primary tumor cm in diameter, pelvic location of the primary tumor, and presence of overt metastatic disease at diagnosis. Patients with metastatic Ewing tumors have a DFS rate of 20% when treated with conventional therapy. Dose intensification with stem cell support has been tried in Ewing sarcoma patients, but several large retrospective studies have failed to show a clear benefit from autologous HCT compared with conventional therapies. In a study from the National Cancer Institute, 91 patients were enrolled in a series of 3 protocols consisting of induction chemotherapy, radiation to the primary site, consolidation with TBI (8 Gy), and autologous HCT. In this group, 79% of the patients achieved a CR with surgery, local radiation, and chemotherapy; 90% of eligible patients proceeded to transplantation; and 30% survived long term without progression of disease. Although this proportion is higher than expected for a poor-prognosis group of patients, this may represent selection of a chemotherapy-sensitive better risk group because only patients who did not progress after chemotherapy were eligible for autologous HCT.

Adoptive cell therapy

Adoptive cell therapy is the transfer of autologous or allogeneic immune cells with direct activity against cancers or infections into a patient. The approach dates back to the first demonstration in the late 1980s that ex vivo expanded autologous populations of tumor-infiltrating lymphocytes could mediate tumor regression in patients with metastatic melanoma. Over the past 2 decades, advances in gene-transfer technologies have enabled efficient redirection of immune cells toward cancer antigens to overcome immune tolerance seen with tumor-infiltrating lymphocytes. To date, most of the progress in adoptive cell therapy has been in hematologic malignancies with engineered T cells expressing chimeric antigen receptors (CARs), with 2 CD19-directed therapies (tisagenlecleucel, axicabtagene ciloleucel) being approved by the US FDA in

2017. Emerging data also support efficacy of T cells with engineered T cell receptors (TCRs) as well as adoptively transferred NK cells. Adoptive cell therapy is also being studied for the treatment of opportunistic infections in immunocompromised patients.

Cancer therapy with chimeric antigen receptor-modified T cells

Adoptive cell therapy with T cells genetically engineered to express a CAR has emerged as a treatment modality for patients with hematological malignancies. In its basic (first-generation) form, a CAR is a recombinant receptor construct consisting of an extracellular single-chain variable fragment of an antibody recognizing a tumor-associated cell surface antigen, a spacer or hinge region, and the TCR CD3 ζ chain without costimulatory domains. Unlike physiologic activation of T cells, this construct leads to activation of the engineered T cell upon contact with the target antigen in an HLA-independent manner. While effective in vitro, first-generation CARs have partial expansion and limited in vivo persistence, yielding limited clinical efficacy. Enhanced in vivo expansion and persistence of CAR T cells is achieved with second-generation CAR constructs that include a costimulatory domain (eg, CD28, 4-1BB, OX40). Even greater activation, proliferation, and efficacy is achieved with third-generation CARs that include 2 costimulatory domains. CAR constructs may be integrated into autologous or allogeneic T cells through lentiviral or retroviral transduction or electroporation of CAR-coding messenger RNA constructs. After ex vivo expansion, T cells are infused into patients following lymphodepleting chemotherapy (typically fludarabine with or without cyclophosphamide), which facilitates in vivo expansion by removal of regulatory T cells and generation of a supportive cytokine milieu.

Adoptive cell therapy with CAR T cells is associated with unique toxicities, which can be severe or even fatal. For CD19-directed therapy (see below), common side effects are cytokine release syndrome (CRS), neurological toxicity, and B-cell aplasia. CRS is the most commonly observed toxicity and thought to be the result of cytokines such as IFN- γ , TNF- α , and IL-6 released from activated lymphocytes and/or other immune cells during the anti-tumor response and rapid CAR T cell activation and expansion. This systemic inflammatory disorder ranges in severity from low-grade constitutional symptoms, such as fever and flu-like symptoms, to a high-grade syndrome associated with hypotension, lung injury, and life-threatening multiorgan dysfunction. Fulminant macrophage activation syndrome or hemophagocytic lymphohistiocytosis may occur. Treatment of CRS is primarily symptomatic/support-

ive and may include the use of vasopressors, blood product transfusions, and mechanical ventilation. The IL-6 receptor antagonist antibody tocilizumab can abrogate CRS without interfering with the antitumor response; whereas systemic corticosteroids, while also effective for the treatment of CRS, may interfere with the antitumor activity of CAR T cells. Neurotoxicity, manifesting as delirium, dysphasia, akinetic mutism, and seizures, is the second most common side effect of CAR T cell therapy and can occur concurrently with or after CRS. Largely reversible, fatal cases of cerebral edema have occurred.

For patients with hematologic malignancies, clinical development has been primarily for CAR T cells targeting CD19. CD19 is an attractive target because of its homogeneous and uniform expression during all stages of B-cell differentiation and malignant transformation and its absence on other cell types. Response rates of approximately 80% to 90% have been reported with CD19-directed CAR T cells in pediatric and adult relapsed ALL. In August 2017, tisagenlecleucel (CTL019) became the first CAR T cell therapy to gain regulatory drug approval by the FDA. Approval was granted based on results from a single-cohort, multicenter global phase 2 trial (ELIANA). Among 75 patients ages 3 to 23 years with relapsed or refractory CD19+ B-ALL who received an infusion of tisagenlecleucel, the overall remission rate was 81% (all measurable residual disease-negative) with 12-month EFS and OS estimates of 50% and 76%, respectively. Grade 3/4 events suspected to be due to the CAR T cell therapy were noted in 55 of the 75 patients (73%). Of note, 77% of the patients experienced cytokine release syndrome, with 48% of them requiring tocilizumab for management, while 40% experienced neurologic events. In October 2017, axicabtagene ciloleucel (axi-cel) became the second approved CAR T cell therapy. The drug gained FDA approval for the treatment of adults with relapsed or refractory large B-cell lymphoma after 2 or more lines of systemic therapy, including DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from FL. Approval was granted based on results from a single-cohort, multicenter phase 2 trial (ZUMA-1). In this study, 101 patients (of 111 enrolled) ages 23 to 76 years with histologically confirmed relapsed or refractory large B-cell lymphoma received a target dose of 2×10^6 CAR T cells/kg body weight after undergoing lymphodepleting chemotherapy with low-dose cyclophosphamide and fludarabine. The overall response rate was 82%, with a complete response rate of 54% and median duration of response of 8.1 months. OS was estimated at 52% at 18 months for patients receiving CAR T cells. Neutropenia, anemia, and thrombocytopenia emerged as the most com-

mon grade 3 or higher events. CRS and neurologic events occurred in 93% and 64% (grade 3 or higher in 13% and 28%) of the patients, respectively.

While clinical data are most mature with CD19-directed CAR T cell therapy, an increasing number of other antigen targets are being pursued as well, including CD20, CD22, CD30, CD33, CD123, and the B-cell maturation antigen (BCMA), among others. Early data with BCMA-directed CAR T cells for patients with relapsed multiple myeloma, suggest that the clinical success of this type of adoptive cell therapy extends beyond CD19. Since targeting single antigens with CAR T cells carries the risk of immune escape or loss of the target antigen, a phenomenon well documented in patients treated with CD19-directed CAR T cells, current studies are also exploring the simultaneous targeting of multiple antigens (eg, CD19 and CD22).

Cancer therapy with T cell receptor–engineered cells

Compared to CAR-modified T cells, adoptive cell therapy with autologous TCR-engineered T cells has garnered less attention as an approach to redirect T cells toward defined cancer antigens. TCR-engineered cells are most effective for the targeting of peptides from tumor-associated cell membrane or intracellular/nuclear proteins as they are presented on the cell surface by HLA molecules. This includes tissue-specific differentiation antigens, cancer-testis antigens, overexpressed antigens, and mutated self-proteins that form neoantigens. This approach depends on the generation of TCR α and β chains specifically recognizing an intended tumor target and expressing engineered TCR molecules in autologous T cells. A number of strategies can be used to identify and obtain appropriate α and β sequences; for example, from isolated patient-derived tumor-reactive T cells, human HLA-bearing mice vaccinated with tumor protein and, in an allo-MHC-restricted approach, lymphocytes found in HLA-A2-negative individuals with high avidity for tumor-associated antigens. Efficient TCR gene transfer can be achieved with retroviral and lentiviral vectors or the nonviral sleeping beauty system, with each modality carrying a risk of insertional mutagenesis. Once introduced, the therapeutic TCR α/β heterodimer then requires noncovalent assembly with CD3 γ , CD3 δ , CD3 ϵ , and CD3 ζ subunits to form a complete TCR-CD3 complex on the cell surface. In contrast to later-generation CAR constructs, current TCR engineering does not involve the introduction of extracellular stimulatory domains, so that gene-modified cells depend on the retention of natural TCR-signaling components for functionality. The ability of TCR-engineered cells to recognize the intended tumor cell depends on the cell surface

abundance of the therapeutic TCR α/β heterodimer, as well as the receptor's affinity for the target antigen, aspects that need optimization (eg, to reduce mispairing with endogenous TCR chains, which could theoretically result in unexpected, self-reactive TCR specificities with potential to cause off-target autoimmunity).

Most experience with TCR-based adoptive cell therapy has been gained in patients with advanced solid tumors. Several small studies have tested TCRs directed at MART-1 and pg100 (metastatic melanoma), MAGE-A family members (primarily metastatic melanoma and esophageal cancer), CEA (colorectal cancer), and NY-ESO-1a (primarily metastatic melanoma and synovial sarcoma). In these studies, patients generally received ex vivo expanded, gene-modified autologous peripheral blood lymphocytes after administration of lymphodepleting chemotherapy (most commonly cyclophosphamide and fludarabine) and in conjunction with IL-2. Together, available data from these trials suggest the potential of TCR-engineered cells to exert clinically significant antitumor efficacy. However, in many cases, tumor responses were of short duration, and further methodological refinements are necessary to increase the in vivo persistence and functionality of these cells to maintain their anticancer effects. The clinical experience is limited in hematologic malignancies, but data from small studies reporting possible antitumor efficacy with the use of autologous T cells expressing TCRs against NY-ESO^{c259} (multiple myeloma) or WT1 (AML) suggest the benefit may extend to some patients with blood cancers as well. Several trials with TCR-engineered cells are currently ongoing and the clinical experience with these cells for the treatment of hematologic malignancies and solid tumors is likely to increase substantially over the next several years. These trials will also clarify the spectrum of associated toxicities. While infusion of ex vivo expanded TCR-modified cells was well tolerated without significant safety concerns or apparent CRS in some trials, others have highlighted the potential of these cells, perhaps particularly when using higher-affinity TCRs, to cause adverse on-target, off-tumor as well as off-target toxicity and substantial morbidity and mortality (eg, inflammatory colitis [CEA], skin rash [MART-1, pg100], and cardiac/neurologic toxicity [MAGE-A]).

T cell therapy for the treatment of viral infections

The frequency of opportunistic viral infections in allogeneic HCT recipients, combined with inadequacies and toxicities of current pharmacological therapies, has raised interest in strategies to prevent or treat these infections and their sequelae and to establish long-term immunological memory.

One approach to accomplish this goal includes adoptive cell therapy with prophylactic or therapeutic infusion of donor-derived or third-party ("off-the-shelf") virus-specific T cells. Several techniques have been established for T cell production that vary in the way antigens are presented and T cells are selected and expanded. The methodologies have evolved rapidly, and solutions have been developed that adhere to good manufacturing practices and overcome limitations identified in early clinical studies. As one example, rather than coculturing T cells and antigen-presenting cells (APCs) loaded with virus-derived peptides, proteins, or viral lysates, overlapping peptide libraries (so-called pepmixes) derived from full-length immunodominant viral proteins are pulsed into donor-derived APCs as immunogens and cultured with T cells. Alternatively, APCs can be genetically engineered to present immunogenic viral peptides to T cells. Both approaches allow the development of multivirus-specific T cells and can be used for the generation of cell product from naïve cord blood lymphocytes. Also in line with good manufacturing practices are direct selection techniques via IFN- γ capture or through multimer-based selection, which allow rapid generation of virus-specific T cells and scalability of products.

Adoptive cell therapy with donor-derived, virus-specific T cells has been developed in many centers and used to prevent and/or treat viral infections. Results from early phase trials demonstrate that such cells are safe and can be highly effective in controlling CMV and EBV infections in HCT recipients, conferring protection in up to 70% to 90% of patients. EBV-specific T cells also have shown remarkable efficacy in the prevention of EBV+lymphomas posttransplant, as well as the treatment of established EBV lymphomas with achievement of sustained complete remissions in the majority of patients. In some patients, failure to respond or loss of response has been associated with the presence of viral strains that possess deletions in immunodominant epitopes or origination of virus-associated tumor cells in recipient rather than donor cells. In recent years, the spectrum of infections targeted with pathogen-specific (or multipathogen-specific) T cells has expanded to include additional viruses seen in immunocompromised patients (eg, adenovirus, VZV, HHV-6, polyomaviruses [BK virus, JC virus, and Merkel cell carcinoma virus]), influenza and fungi (eg, *Aspergillus*).

Third-party products from banks of cryopreserved virus-specific T cells offer readily available therapy that can overcome the need for patient-specific T cell manufacturing. Closely matched third-party products yielded responses in up to 70% of patients with resistant CMV, EBV, or adenovirus infection. However, while third-party

products do not require full HLA matching to the recipient for anti-viral activity, identification of closely-matched products can be difficult, and suboptimal HLA matching has been associated with lack of T cell expansion in recipients. These products also have the theoretical concern of alloreactivity but an increased risk of GVHD has so far not been observed clinically.

Natural killer cells

As reviewed elsewhere in this book in greater detail, NK cells are part of the innate immune system and can exert antitumor and antimicrobial activity in an antigen-independent fashion. This activity is modulated by an intricate balance between various activating and inhibitory receptors, including the killer cell immunoglobulin-like receptors. Unlike T cells, NK cells do not require prior antigen sensitization to elicit cytotoxic effects and do not cause GVHD in the allogeneic setting, properties that render them very attractive for adoptive cell therapy. Early studies demonstrated that autologous NK cells could be expanded and activated ex vivo (eg, with cytokines) but the use of high-dose IL-2 given to patients together with infused cells led to unacceptable toxicities. The tolerance of this approach could be improved, and safety demonstrated, once low-dose IL-2 was used. However, even though some complete remissions were noted in patients with metastatic solid tumors, outcomes remained suboptimal.

More recent efforts have focused on allogeneic NK cells. In this setting, enrichment of NK cells collected from the peripheral blood is usually achieved by depletion of T- and B cells, with or without additional positive selection of CD56-positive cells to enrich for NK cells. To increase the number of NK cells and improve their antitumor activity, a variety of protocols have been developed for the ex vivo expansion/activation of cells from healthy donors. Infusions of haploidentical NK cells that have undergone short- or long-term activation or expansion have demonstrated antitumor efficacy in patients with AML or multiple myeloma, with a low rate of rejection and side effects. Lymphodepleting conditioning chemotherapy with cyclophosphamide and fludarabine can facilitate NK cell persistence and expansion in vivo, possibly in part because it leads to high production of IL-15. Currently, efforts are ongoing to overcome limitations of autologous NK cells by combination with NK cell engaging bi- or trispecific molecules or additional cytokines (eg, IL-15) and to explore sources other than the peripheral blood for NK cells such as human embryonic stem cells, induced pluripotent stem cells, or umbilical cord blood. Another effort explores the modification of NK cells to improve their persistence, cytotoxicity, and hom-

ing. Examples of modifications include the introduction of CD16a (to increase ADCC effects) and genetic engineering to produce cytokines (eg, IL-15 and IL-2) or to express CAR constructs targeting a variety of tumor-associated antigens (eg, CD5, CD7, CD19, CD20, CD33, and CD138). Similar to constructs used for T cells, second- and third-generation CAR constructs designed for NK cells contain cell-specific signaling endodomains such as 4-1BB, DAP-12 or 2B4 (CD244). Unlike with CAR T cells, however, studies with CAR-modified NK cells are just entering clinical trials.

Summary

HCT is a rapidly evolving field. Results have improved over the past decades, and indications for HCT continue to expand and change. Transplantation is more widely applicable because of improvements in supportive care and donor selection and the advent of NMA and reduced-intensity conditioning regimens. For patients with malignant diseases, the chance for a better outcome is significantly improved if they are referred early when their disease still demonstrates chemotherapy sensitivity. For most of the other indications, it is important to identify high-risk features or poor prognostic factors at the time of diagnosis to help determine the optimal timing for HCT. Adoptive cell therapy targeting cancer-associated antigens, while still relatively early in development, is adding a powerful new therapy to existing therapies and is beginning to revolutionize care of patients with hematologic malignancies. Antiviral T cell therapies also hold promise to reduce NRM after allogeneic HCT, potentially improving HCT outcomes.

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16

Inherited marrow failure syndromes and myeloid disorders

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Introduction

Bone marrow (BM) failure refers to the inability of BM hematopoiesis to meet physiologic demands for *production* of healthy blood cells due to dysfunction or loss of hematopoietic stem or progenitor cells (HSPC). Pancytopenia with reduced red cells, neutrophils and platelets, or bi- or unilineage cytopenias may result. Lymphocyte numbers are usually preserved, due to self-renewal abilities of mature T cells. Initial differential diagnosis of cytopenias first requires distinguishing production defects from peripheral destruction, consumption, or blood loss. Besides BM examination, reduced numbers of reticulocytes and/or immature platelets, and/or an elevated MCV, may suggest a BM failure syndrome. BM failure syndromes can be classified into acquired idiopathic, inherited, iatrogenic or environmental (ie, radiation or chemotherapy), or due to vitamin/nutrient deficiency. Acquired BM failure states, including aplastic anemia and myelodysplasia, are discussed in chapter 19.

The range of molecular mechanisms responsible for inherited BM failure states (discussed in this chapter) is broad, including abnormal DNA-damage response (Fanconi anemia [FA]), defective ribosome biogenesis (Diamond-Blackfan anemia [DBA] and Shwachman-Diamond syndrome [SDS]), defective telomere maintenance (dyskeratosis congenita [DC] and other telomere biology disorders termed “telomeropathies”), and altered hematopoietic growth factor

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Off-label drug use: Dr. Bertuch and Dr. Dunbar: androgens (eg, oxymetholone or danazol) in Fanconi anemia and telomeropathies; G-CSF in non-SCN neutropenia; glucocorticoids in Diamond-Blackfan anemia, autoimmune neutropenias, and macrophage activation syndrome; iron chelating agents in congenital dyserythropoietic anemias; interferon- α in congenital dyserythropoietic anemia type I and Erdheim-Chester disease; plerixafor in WHIM syndrome; intravenous immunoglobulin in autoimmune neutropenias; interferon- γ in chronic granulomatous disease; colchicine in familial Mediterranean fever; anakinra in Majeed syndrome; etoposide, methotrexate, cyclosporine, antithymocyte globulin and dexamethasone in hemophagocytic lymphohistiocytosis; topical steroids, nitrogen mustard, psoralen in Langerhans cell histiocytosis; vinblastine, methotrexate, and glucocorticoids in Langerhans cell histiocytosis.

receptor–kinase signaling (congenital amegakaryocytic thrombocytopenia [CAMT]). In some inherited marrow failure syndromes, the mechanism of hematopoietic failure is currently unclear. We focus primarily on those marrow failure states presenting primarily with hematologic manifestations and cared for by hematologists, omitting some rare fatal genetic disorders presenting in infancy or childhood with severe neurologic and/or multisystem failure along with cytopenias.

The term *myeloid* derives from the Greek *myelos*, meaning “marrow,” and in its broadest sense is used to describe hematologic conditions or diseases originating in the BM. *Myeloid* is also used more narrowly to describe disorders primarily involving granulocytes (neutrophils, eosinophils, or basophils) and monocytes, as opposed to other cell lineages such as lymphoid cells. A variety of myeloid disorders are also described in this chapter, including inherited and acquired neutropenias, neutrophilia, neutrophil function abnormalities, acquired and inherited histiocytic and autoinflammatory disorders, and macrophage storage disorders.

Granulocytes: neutrophils, eosinophils, and basophils

The term *granulocytes* refers to circulating neutrophils, eosinophils, and basophils; although because of neutrophil predominance in the blood, the terms *neutrophil* and *granulocyte* are sometimes used synonymously. Normal values for the differential counts of leukocytes in the blood vary with age, ethnicity, and laboratory. Neutrophils are a critical component of the innate immune response, and persistent neutropenia is associated with a marked susceptibility to bacterial and fungal infections. Conversely, neutrophils are also a major contributor to tissue damage in inflammatory diseases. Neutrophil homeostasis in the blood is regulated at 3 levels: neutrophil production in the BM, neutrophil release from the BM to blood, and neutrophil clearance from the blood (Figure 16-1).

Neutrophils

Neutrophil production

Under normal conditions, neutrophils are produced exclusively in the BM, where it is estimated that 10^{12} are generated on a daily basis. The primary driver of neutrophil production is the cytokine granulocyte colony-stimulating factor (G-CSF). Neutrophilic differentiation from multipotent HSPC is regulated by the coordinated expression of a number of key myeloid transcription factors, including CCAAT enhancer-binding proteins α (C/EBP α), C/EBP ϵ ,

and GFI-1. A number of hematopoietic growth factors provide extrinsic signals that regulate various stages in HSPC differentiation to myeloid lineages, including neutrophils. G-CSF stimulates the proliferation of precursors, reduces the average transit time through the precursor compartment, mediates neutrophil release from the BM, and prevents apoptosis of mature cells.

Neutrophil release

Neutrophils are released from the BM into the blood in a regulated fashion to maintain homeostatic levels of circulating neutrophils. The BM provides a large reservoir of mature neutrophils that can be mobilized readily in response to infection or inflammation. A broad range of substances have been shown to induce neutrophil release from the BM, including chemokines, cytokines, microbial products, and various other inflammatory mediators. The chemokine stromal derived factor-1 (SDF1, also termed CXCL12) and the cognate chemokine receptor CXCR4 play a key role in retaining a pool of neutrophils in the BM, whereas the chemokine receptor CXCR2 and its ligands play a role in their release.

Neutrophil clearance

Neutrophil homeostasis in the blood is determined, in part, by the rate of clearance from the circulation. Once released into the circulation, neutrophils have been thought to have a short half-life of only 5 to 6 hours, however the most recent studies suggest some neutrophils may persist in the circulation for up to 5 to 6 days. Neutrophils are cleared primarily in the liver, spleen, or BM, where apoptotic or aged neutrophils are phagocytosed by macrophages.

Neutrophil margination and tissue extravasation

Neutrophils in the circulation loosely attach and subsequently adhere to vascular endothelium in response to the local production of inflammatory cytokines and chemokines, a process termed margination (Figure 16-1). Normally, approximately one-half of the neutrophils in the circulation are in this marginal pool. The other half circulates freely. Selectins mediate neutrophil rolling and β_2 -integrins mediate firm adherence and vascular transmigration. Indeed, deficiency of selectin ligands or β_2 -integrins causes leukocyte adhesion deficiency (see below).

Once recruited to an infected tissue site, neutrophils serve phagocytic, immunomodulatory, and remodeling functions. Surface receptors for immunoglobulins and complement enhance ingestion and killing of microorganisms. Within the cell, the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system, enzymes found in the cell’s primary and secondary granules, and cytoplasmic

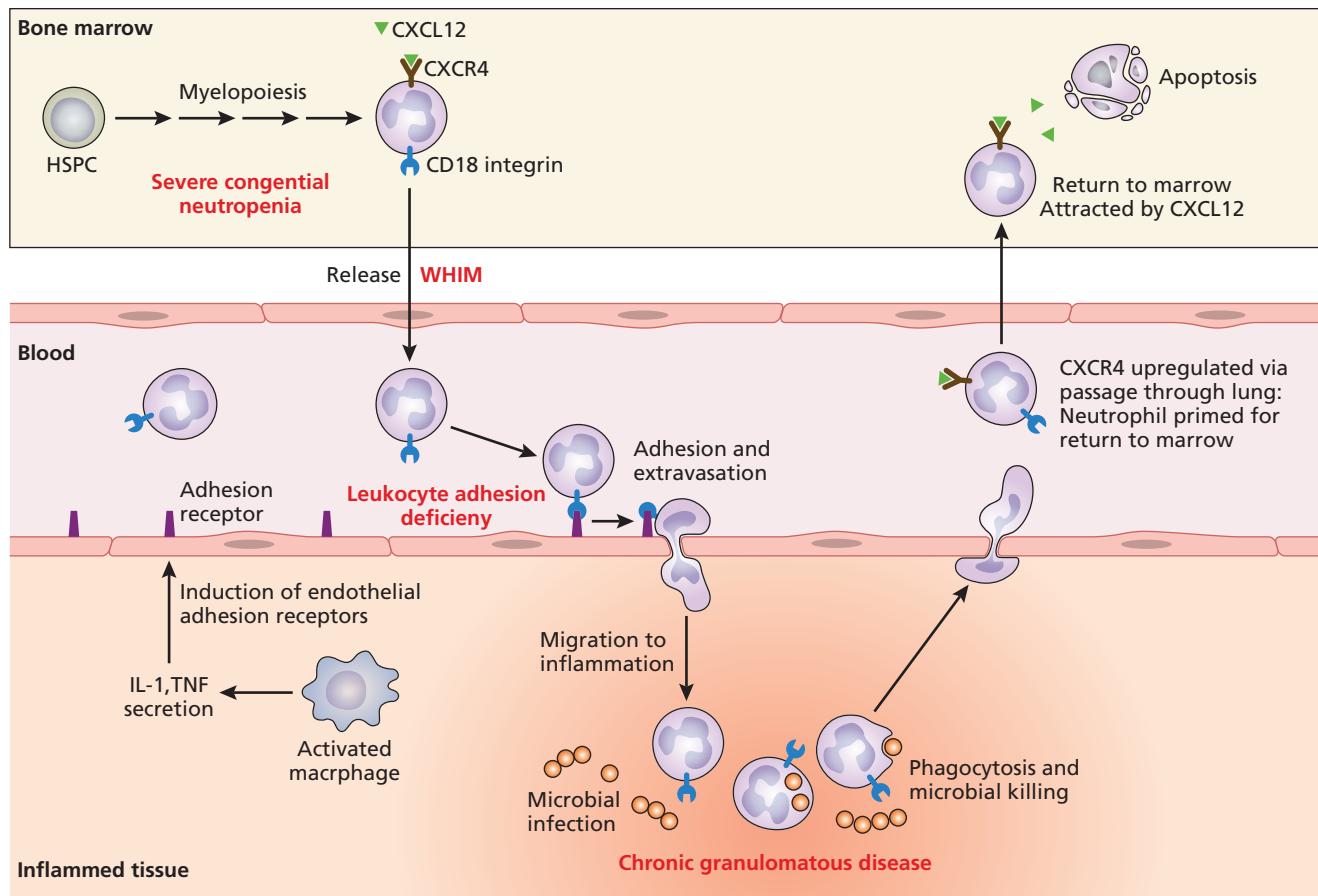


Figure 16-1 The neutrophil life cycle and inherited myeloid disorders. Red text indicate congenital disorders linked to defects in various stages in the neutrophil life cycle. The BM is the primary site of myeloid development in humans. In SCN, neutrophil development in the marrow is blocked prior to completion. Under basal conditions, the BM retains a large reservoir of mature neutrophils, mediated primarily via CXCL12 ligand/CXCR4 receptor interactions. In WHIM syndrome, a hyperactive CXCR4 receptor on neutrophils results in an increase in neutrophil retention in the marrow (myelokathexis). In response to tissue inflammation, circulating neutrophils adhere to endothelium via CD18 integrin binding to adhesion molecules on endothelial cells induced by inflammatory mediators such as IL-1. Neutrophils then diapedese through the endothelium and migrate along chemokine gradients to reach the site of inflammation. In LAD type 1, lack of functional CD18 results in lack of adhesion and defective tissue entry. Once neutrophils reach the site of inflammation, phagocytosis of bacteria and fungi occurs. In CGD, mutations in components of the NADPH oxidase complex result in impaired microbial killing. Neutrophils return to the circulation from tissues, upregulate CXCR4 following transit through the lung, and home back to the BM in response to a CXCL12 gradient, resulting in apoptosis and clearance of senescent neutrophils.

glycogen are involved in the intracellular oxidative burst that accompanies phagocytosis and the killing and digestion of microorganisms. These pathways are critical for normal host defense mechanisms, and mutations in each can result in diseases characterized by enhanced susceptibility to infection, such as chronic granulomatous disease (CGD) (see below). Neutrophils also infiltrate tumors and have been associated with both antitumor and protumor effects. Until recently, neutrophil egress from the circulation was considered to be one-way, but imaging and tracking studies now document return back to the circulation en route to the lungs, followed by apoptosis in the BM.

Neutrophilia

Neutrophilia is an excess of circulating neutrophils and is typically defined as an *absolute neutrophil count* (ANC) >2 standard deviations above the mean, which in adults corresponds to an ANC of $>7,700/\mu\text{L}$. Neutrophilia is associated with a wide variety of normal physiologic conditions, responses to stress, and benign and neoplastic disorders (Table 16-1). A prompt increase in the blood neutrophil count, as well as the circulating levels of other leukocytes, occurs with acute stress, exercise, anxiety, and some drugs—most notably corticosteroids or epinephrine. Only rarely does this response more than double the count. These factors generally

Table 16-1 Causes of neutrophilia

Acute neutrophilia	Chronic neutrophilia
Acute infections	Chronic infections
Many localized and systemic acute bacterial, mycotic, rickettsial, spirochetal, and certain viral infections	Fungal and mycobacterial
Inflammation or tissue necrosis	Inflammation
Burns, electric shock, trauma, myocardial infarction, gout, vasculitis, antigen-antibody complexes, complement activation	Continuation of most acute inflammatory reactions, such as rheumatoid arthritis, gout, chronic vasculitis, myositis, nephritis, colitis, pancreatitis, dermatitis, thyroiditis, drug-sensitivity reactions, periodontitis, Sweet syndrome, familial periodic fever syndromes
Physical or emotional stimuli	Tumors
Cold, heat, exercise, convulsions, pain, labor, anesthesia, surgery, severe stress	Any tumors, but especially gastric, lung, breast, renal, hepatic, pancreatic, uterine, and squamous cell cancers
Drugs, hormones, and toxins	Drugs, hormones, and toxins
Epinephrine, etiocholanolone, endotoxin, glucocorticoids, venoms, vaccines, colony-stimulating factors, rebound from drug-induced agranulocytosis, repletion therapy of megaloblastic anemias	Cigarette smoking, continued exposure to many substances that produce acute neutrophilia; lithium; rarely, as a reaction to other drugs
	Metabolic and endocrinologic disorders
	Pregnancy and lactation, eclampsia, thyroid storm, Cushing disease
	Hematologic disorders
	Chronic hemolysis or hemorrhage, asplenia, myeloproliferative disorders, overlap myelodysplastic/myeloproliferative disorders
	Hereditary and congenital disorders
	Down syndrome, familial Mediterranean fever, leukocyte adhesion deficiency, hereditary neutrophilia
	Chronic idiopathic neutrophilia

increase circulating neutrophils due to demargination of cells from vessel walls, not to the release from the marrow.

Neutrophilia associated with infections and inflammatory disorders occurs by 2 general mechanisms. First, during infection, a number of inflammatory cytokines are released into the circulation that induces the release of mature neutrophils from the BM. Second, the sustained cytokine and inflammatory response associated with infections stimulates neutrophil production in the BM. In contrast to neutrophil demargination, neutrophilia associated with infections and inflammatory disorders is marked

by the presence of an increase in immature granulocytes in the blood, including band forms and occasionally metamyelocytes and earlier precursors. In addition, there often is a change in the morphology of neutrophils, with appearance of vacuoles and more intensely staining “toxic granulations”. Cells released prematurely also may contain bits of endoplasmic reticulum that stain as blue bodies in the cytoplasm, called Döhle bodies (Figure 16-2).

In most cases of reactive neutrophilia, the inciting infection (or other stress) is usually clinically obvious, and neutrophilia is self-limited. In patients without demonstration

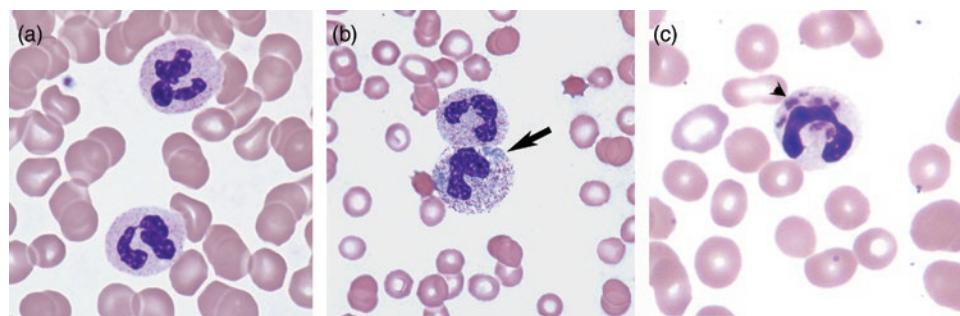


Figure 16-2 Photomicrographs of blood smears showing typical neutrophil morphology from (a) a healthy individual; (b) a patient with sepsis showing Döhle bodies (arrow) and toxic granulations; (c) a patient with CHS showing large cytoplasmic inclusions (arrowhead). ASH Image Bank images 3780 (a), 3778 (b), 2979 (c).

of a clonal marker by either cytogenetic or molecular testing, clinical features such as the presence of splenomegaly, leukoerythroblastic features on the blood smear (teardrop and nucleated red blood cells), basophilia, or circulating promyelocytes or blasts are highly suggestive of an underlying myeloproliferative disorder (MPD) or MPD/myelodysplastic syndrome (MDS). See Chapter 18 for information on neutrophilia linked to MPD or MPD/MDS disorders. In some cases, chronic neutrophilia may result from inherited intrinsic defects of neutrophil function or trafficking or from inflammatory syndromes as presented in detail later in this chapter.

Neutropenia

Neutropenia is commonly defined as an ANC of <1,500 cells/ μ L. Neutrophil levels can be lower in healthy individuals from some ethnic and racial groups (eg, Africans, African-Americans, Caribbean-Americans and Yemenite Jews) as compared to those of European descent (Table 16-1) and is termed benign ethnic neutropenia (BEN). Up to 4% of African-Americans have BEN, with no apparent increase in infections. Baseline lower neutrophil counts may impact on eligibility for cancer clinical trials or chemotherapy dose intensity. Systems controlling steady-state neutrophil numbers are poorly understood.

Neutropenia is classified based on the ANC as severe (<500/ μ L), moderate (500 to 1,000/ μ L), or mild (1,000 to 1,500/ μ L). The risk of infection increases empirically when the ANC falls below 500/ μ L; however, risk of the most serious infections rises sharply with ANC <200/ μ L. Patients with neutropenia are prone to develop bacterial infections, typically caused by endogenous flora and involving mucous membranes, including gingivitis, stomatitis, perirectal abscesses, cellulitis, and pneumonia. Fungal infections are a major cause of mortality in patients with chronic severe neutropenia. There is no increase in susceptibility to viral or parasitic infections with isolated neutropenia. It is important to take into account whether neutrophil counts are falling or recovering, and whether neutrophil function itself may also be impaired (eg, in MDS) when assessing the clinical risk of neutropenia.

The differential diagnosis of neutropenia is broad (Table 16-2). Neutropenia is a frequent manifestation of MDS, acute leukemia, autoimmune disorders, and marrow-infiltrative processes such as myelofibrosis, or metastatic carcinoma; these are discussed in detail in their respective chapters.

Eosinophils and basophils

Marrow HPSC produce a small proportion of eosinophils and basophils. The granules of eosinophils contain histamine and proteins important for the killing of para-

Table 16-2 Causes of neutropenia

Inherited neutropenia syndromes*

Severe congenital neutropenia (sometimes termed Kostmann syndrome)

Cyclic neutropenia

Shwachman-Diamond syndrome

WHIM syndrome (myelokathexis)

Chédiak-Higashi syndrome and other disorders of vesicular transport

Pearson syndrome (+/– anemia)

GATA2 deficiency (isolated neutropenia rare)

Fanconi anemia (isolated neutropenia rare)

Dyskeratosis congenita/telomere biology disorders (isolated neutropenia rare)

Acquired neutropenia

Neonatal alloimmune neutropenia

Primary autoimmune neutropenia

Secondary autoimmune neutropenia

Systemic lupus erythematosus

Felty syndrome

Nutritional deficiencies (vitamin B₁₂, folic acid, copper)

Myelodysplastic syndromes

Acute leukemias

Myelophthysis (BM infiltration by tumor, fibrosis, granulomas)

Large granular lymphocytic leukemia

Neutropenia associated with infectious disease

Sepsis

Rickettsial: human granulocytic ehrlichiosis

Viral: mononucleosis, HIV

Drug-induced neutropenia

Hypersplenism

*Not restricted to disorders in which neutropenia is the only manifestation.

sites. Eosinophil production is increased and eosinophilia ($>1.0 \times 10^9/L$) occurs in allergic disorders (eg, asthma, allergic rhinitis, dermatitis), parasitic infections, collagen vascular diseases, and drug reactions. Paraneoplastic eosinophilia can result from release of interleukin-5 (IL-5) by lymphoma cells. Myeloproliferative hypereosinophilic syndromes can result from translocations activating tyrosine kinase receptors, particularly platelet-derived growth factor receptors A and B and can be effectively treated with tyrosine kinase inhibitors. Hypereosinophilia to levels $>15 \times 10^9/L$ can result in end-organ damage, particularly to the heart and lungs.

Basophils are the least numerous blood leukocytes. Basophilic granules contain histamine, glycosaminoglycans,

major basic protein, proteases, and a variety of other vasoactive inflammatory mediators. Basophils primarily function to activate immediate (type 1) hypersensitivity responses. Basophilia is associated with hypersensitivity reactions, including drug and food allergies. Basophilia is a common feature of MPDs, particularly chronic myeloid leukemia (CML), and can aid in diagnosis of these disorders. Basophilia also can be associated with other chronic inflammatory diseases, such as tuberculosis, ulcerative colitis, and rheumatoid arthritis, but is rarely seen as an isolated finding.

Monocytes and related cells

Monocytes and related histiocytes and macrophages serve antimicrobial, scavenger, and secretory functions, and also participate in tissue repair and antigen processing and presentation. Monocytes serve as precursors for both circulating macrophages and tissue-associated cells related to macrophages. These cells are often associated with the endothelium, particularly in the spleen and liver, where they clear microorganisms and aged or damaged blood cells from the circulation. Alveolar macrophages in the lung, Kupffer cells in the liver, sinus histiocytes in lymph nodes, Langerhans cells in the skin, microglia in the central nervous system, and osteoclasts in the bone are all forms of tissue histiocytes or macrophages that are thought to derive from blood monocytes. These tissue populations may be very long-lived, populated originally from monocytes or monocyte precursors migrating to tissues during fetal development.

Monocytes and related cells are a primary source of the inflammatory cytokines (eg, tumor necrosis factor, interleukin-1, interferons) that cause fever and many of the symptoms associated with infections or inflammation. There are 2 types of activated tissue macrophages: type 1 produces interleukin-12 in response to bacterial products or interferons and is proinflammatory and primarily involved in response to pathogens, and type 2 instead is involved in tissue repair and produces anti-inflammatory cytokines such as interleukin-10. Chronic or dysregulated stimulation of tissue macrophages may contribute to acquired or inherited systemic inflammatory syndromes. Type 2 macrophages may play a central role in the ability of tumors to evade the immune system.

Dendritic cells share a precursor with monocytes and consist of several subtypes of cells that participate in both innate and adaptive immune responses. These cells are distributed widely throughout virtually all tissues, particularly concentrated in lymphoid tissues associated with barriers, such as the skin and mucosal surfaces. A major function of dendritic cells is to process and present antigens to T cells. Immature dendritic cells in the peripheral tissue

express surface receptors that allow them to recognize and take up extracellular antigens in their environment, stimulating activation, maturation, and migration to secondary lymphoid tissues, where they bind and present antigen in the context of major histocompatibility complex class I and II molecules to stimulate naive T cells. Mature dendritic cells also produce cytokines important in priming T cells. Specific dendritic cell subsets have been identified and characterized based on their location, cell surface phenotype, function, or developmental stage. The ability of dendritic cells to present tumor antigens has led to their use in vaccine immunotherapy trials.

Moncytosis

Monocytes normally account for approximately 1% to 9% of peripheral blood leukocytes, with absolute monocyte counts ranging from 0.3×10^9 to $0.7 \times 10^9/L$. An increase in circulating monocytes may be observed in chronic inflammatory conditions and chronic infections, such as tuberculosis, endocarditis, and syphilis. In inflammatory conditions, monocytosis is a reactive process resulting from the peripheral production of cytokines, which stimulate monocyte production. Moncytosis is a hallmark of the MPD/MDS overlap syndrome chronic myelomonocytic leukemia and of the pediatric disorder juvenile myelomonocytic leukemia (JMML). It may also be observed in association with lymphomas and acute monocytic (monoblastic) leukemias. Malignant monocytosis is presumed to be due to specific molecular defects affecting monocyte proliferation, differentiation, and survival.

Moncytopenia

Transient moncytopenia occurs with stress, various infections including overwhelming sepsis, and as the result of cytotoxic chemotherapy. A decreased absolute monocyte count can be encountered in acquired BM failure states such as aplastic anemia (AA) and, less commonly, MDS. Monocyte numbers are suppressed, but circulating monocyte counts and function are maintained in many other conditions that cause neutropenia. Moncytopenia in association with natural killer (NK) cell deficiency and B cell lymphopenia can be part of the spectrum of disorders linked to mutations in *GATA2* or *SAMD9L* (discussed later in this chapter). Moncytopenia, along with neutropenia, is characteristic of hairy cell leukemia.

Inherited marrow failure syndromes

Although the inherited BM failure syndromes are rare disorders, collectively affecting just several dozen new patients in the United States each year, a diagnosis with one of these syndromes has profound implications for medical

management and treatment. Moreover, the diagnosis of an inherited BM failure syndrome in a child may have implications for disease risk in the parents (eg, the telomere biology disorders). Several inherited BM failure syndromes are compared in Table 16-3. As detailed in the table, BM failure is often not the only feature of an inherited BM failure syndrome, and skin, nail, musculoskeletal, urogenital or other phenotypic abnormalities can sometimes help guide diagnosis. Marrow failure may even be absent, or present much later than other clinical manifestations in some patients. Clinical presentations and disease severity may vary significantly between patients, even with the same disorder, due to differences in specific mutations, modifying genes, or environmental factors. Even syndromes eventually resulting in pancytopenia can present with a single lineage cytopenia, at times obscuring the underlying diagnosis.

A careful family history is important to elicit in any patients presenting with BM failure or isolated cytopenias, particularly those less than 40 years of age, regarding any family members with cytopenias, blood cancers, and common associated nonhematologic pathologies (eg, liver or lung fibrosis for the telomere biology disorders). Several academic medical centers and commercial laboratories now offer diagnostic targeted sequencing panels that include genes linked to known inherited BM failure syndromes. Application of these panels on cohorts of patients with AA is uncovering germline mutations in patients not previously suspected to have an inherited BM failure syndrome, underscoring the challenge in ruling out these syndromes on clinical features alone.

Fanconi anemia

CLINICAL CASE

A 12-year-old boy presents to his primary care physician with pallor and bruising. His past medical history is remarkable only for an orchiopexy during the first year of life to correct an undescended testis. Pancytopenia is now noted. The patient and his parents do not report any medication or toxin exposures. There are no siblings. On initial examination, the boy appears to be a normal prepubescent male. On closer examination, however, his thumbs appear underdeveloped, and patches of cutaneous hyperpigmentation are noted on his trunk. BM aspiration and biopsy are performed. The marrow cellularity is only 10%; the marrow aspirate shows hypocellular fragments and rare megakaryocytes, most of which are abnormal uninucleate forms. Cytogenetic studies are normal. Exposure of peripheral blood mononuclear cells to diepoxybutane (DEB) results in numerous chromosomal breakages, confirming a diagnosis of FA.

Epidemiology

FA is one of the most common of the inherited BM failure syndromes (common is a relative term here, as the incidence of FA in the United States has been estimated at approximately 1 in 130,000 live births). It is more common among persons of Ashkenazi Jewish descent than among others.

Pathophysiology

A hallmark of cells from patients with FA is hypersensitivity to DNA damage induced by DNA-cross-linking agents, such as DEB and mitomycin C (MMC). FA is a heterogeneous disease at the molecular level, with 21 FA genes identified to date, all encoding factors implicated in a complex DNA repair pathway known as the FA/BRCA pathway (Figure 16-3). Each of these genes, when biallelically mutated, can cause FA, except for *FANCB*, which causes X-linked recessive disease, and *FANCR/RAD51*, which has been associated with autosomal dominant disease. More than 75% of patients have mutations in *FANCA* or *FANCC*.

Numerous studies have clearly established a defect in the ability to repair certain types of DNA damage as the underlying abnormality in FA, although the precise molecular mechanisms are still being elucidated. An important advance has been the finding that the FA pathway is critical for the protection from endogenous aldehyde-induced DNA damage.

Despite progress in identifying the genetic and biochemical defects in FA, it is not known precisely why affected individuals develop BM failure or are at risk for development of clonal hematopoietic neoplasms, including MDS and acute myeloid leukemia (AML). The cause of the progressive AA in FA has been thought to be due to the loss of HSCs because of cumulative DNA damage. Recent studies suggest an exacerbated p53/p21 DNA-damage response impairs hematopoietic stem and progenitor cells in patients with FA. There is also emerging evidence for dysregulation of the transforming growth factor (TGF)- β and tumor necrosis factor (TNF)- α pathways. Additionally, FA hematopoietic progenitor cells are hypersensitive to interferon- γ , a known inhibitor of hematopoiesis.

Clinical features and diagnosis

FA is characterized by pancytopenia and congenital anomalies in the cutaneous, musculoskeletal, cardiac, and urogenital systems. Characteristic physical findings include short stature, microcephaly, intense patchy brown pigmentation of the skin (café au lait spots), and radial ray defects. Hemoglobin F levels are increased in FA, and 80% of patients develop signs of BM failure by age 20 years. Approximately 30% of patients with FA lack typical physical findings, and isolated marrow failure or development of

Table 16-3 The inherited marrow failure syndromes

Genetics, inheritance, and most common genes	Screening test	Nonhematological cancers	Hematological features	Somatic features	Median age at diagnosis (y)	% diagnosed >15 y	Male: female ratio	Syndrome
Fanconi anemia (FA)	Increased chromosome breakage in cells cultured with DNA cross-linking agents (DEB and MMC)	Solid tumors (head, neck, gynecologic, liver, CNS)	Pancytopenia, hypocellular BM, MDS, leukemia	Skin hyperpigmentation and café au lait spots; short stature, triangular face, abnormal thumbs/radii, microcephaly, abnormal kidneys, decreased fertility	6.6	9	1.2:1	Fanconi anemia
Dyskeratosis congenita (DC) and related telomere biology disorders (TBDs)	X-linked recessive, autosomal dominant, autosomal recessive <i>DKC1</i> , <i>TINFE2</i> , <i>TERT</i> , <i>TERC</i> , and <i>RTEL1</i> account for ~60% of cases	Very short telomere length	Solid tumors (head and neck)	Nail dystrophy, abnormal skin pigmentation, leukoplakia, laryngeal duct stenosis, pulmonary fibrosis, liver fibrosis, esophageal strictures, early gray hair, osteoporosis, cerebellar hypoplasia, retinopathy, hypogonadism, urethral stricture	15	46	2:1	Dyskeratosis congenita (DC) and related telomere biology disorders (TBDs)
Diamond-Blackfan anemia (DBA)	Elevated red cell adenosine deaminase (ADA)	Solid tumors, (osteosarcoma, colon), MDS	Macrocytic anemia, occasionally other cytopenias, erythroid hypoplasia in marrow, MDS, leukemia	Short stature, abnormal thumbs, hypertelorism, cardiac septal defect, cleft lip or palate, short neck, hypertelorism, cardiac septal defect, cleft lip or palate, short neck	0.25	1	1.1:1	Diamond-Blackfan anemia (DBA)
Shwachman-Diamond syndrome (SDS)	Low pancreatic isoamylase (after age 3 y) and trypsinogen (before age 3 y); low fecal elastase	None	Neutropenia, anemia, thrombocytopenia AA, MDS, leukemia	Short stature, exocrine pancreatic insufficiency with malabsorption	1	5	1.5:1	Shwachman-Diamond syndrome (SDS)
Severe congenital neutropenia (SCN)	BM exam for promyelocyte arrest	None	Neutropenia, MDS, leukemia	None	3	13	1.2:1	Severe congenital neutropenia (SCN)
Congenital amegakaryocytic thrombocytopenia (CAMT)	BM exam for megakaryocytes	None	Thrombocytopenia; decreased megakaryocytes initially, later AA; MDS, leukemia	Usually none	0	0	0.8:1	Congenital amegakaryocytic thrombocytopenia (CAMT)
Thrombocytopenia absent radii syndrome (TAR)	BM exam for megakaryocytes	None	Thrombocytopenia, MDS, leukemia	Absent radii, abnormal ulnae or humeri (phocomelia), thumbs present, occasional cryptorchidism, hypertension, horseshoe kidney, hemangiomas, micrognathia, cow's milk allergy, cardiac anomalies	0.6	0	0.7:1	Thrombocytopenia absent radii syndrome (TAR)
								Autosomal recessive <i>RBMSA</i>

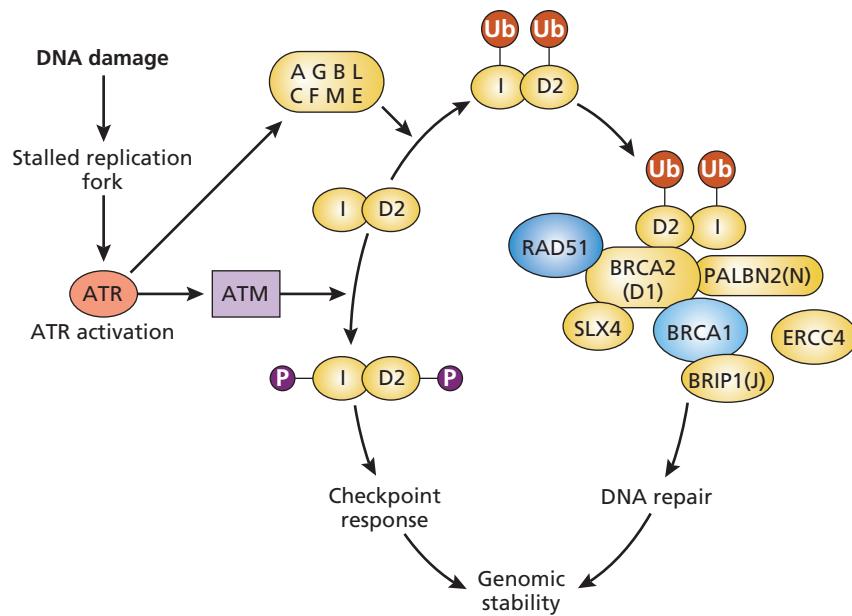


Figure 16-3 A model of the FA pathway. The FA core complex consists of 8 FA proteins (A, B, C, E, F, G, L, and M) and this together with ATR (ataxia-telangiectasia and RAD3 related protein) is essential for the ubiquitination-activation of I-D2 complex after DNA damage. Activated I-D2-Ub translocates to DNA repair foci where it associates with other DNA damage response proteins, including BRCA2 and RAD51 and participates in DNA repair. The proteins mutated in different FA subtypes are shaded yellow.

a malignancy may be the first clinical manifestation of FA. Approximately 10% of patients with FA first come to clinical attention as young adults.

Chromosome breakage testing secures the diagnosis in most patients and entails analysis of chromosome aberrations (breaks and complex rearrangements known as radials) in phytohemagglutinin-stimulated peripheral blood lymphocytes cultured with and without DNA cross-linking agents (eg, DEB or MMC). Results usually are reported as percentage of cells with chromosome aberrations alongside results of cells from a healthy donor (negative control) and from an individual affected by FA (positive control). The percentage of such cells inducible in samples from healthy individuals depends on the specific laboratory protocol but is increased dramatically in FA. BM cells should not be used for chromosome breakage studies because false-negative results are more likely.

Diagnosis of FA may be complicated by the development of somatic mosaicism in lymphocytes. Somatic mosaicism results from a genetic reversion (via recombination or second site repressors) of a mutant *FANCI* gene allele to normal (non-FA), such that a subset of lymphocytes no longer exhibits increased chromosomal breakage in response to DEB or MMC. Because reversion to wild-type confers a growth advantage over the nonreverted FA cells, the diagnosis of FA may be missed. In patients for whom there is a strong suspicion for FA, the diagnosis may be made by testing for chromosomal breakage in response to DEB or MMC using cultured skin fibroblasts obtained from a punch biopsy.

Complications of marrow failure are the most common causes of death in FA, but FA is also characterized by an increased incidence of malignancies. Approximately 10% to 15% of patients with FA develop MDS or AML, often in the context of a hypoplastic marrow and monosomy 7. Patients with FA are also at increased risk for squamous cell carcinomas, in particular head and neck, esophageal, and vulvar/vaginal tumors. In addition to hepatocellular carcinoma, peliosis hepatis and hepatic adenomas occur with increased frequency, especially in patients treated with androgens. The risk of AML is 700-fold in patients with FA compared with the general population but plateaus after the second decade of life, whereas the risk of solid tumors increases with age; in a competing risk analysis, 30% of patients with FA develop a solid tumor by age 48 years.

The clinical significance of an abnormal marrow cytogenetic clone (eg, monosomy 7) in the absence of morphologic dysplasia is not always clear, because these clones may be stable or even regress with time. The exquisite sensitivity of patients with FA to the DNA-damaging effects of chemotherapy and radiation poses a formidable obstacle to the treatment of malignancies in these patients. The most successful treatment of solid tumors in FA results from early detection and complete surgical excision. For this reason, regular tumor surveillance is an important aspect of medical management beginning in the late teenage years.

Treatment

The only potentially curative option for BM failure in patients with FA is allogeneic hematopoietic stem cell

transplantation (HSCT). Because of the increased sensitivity of FA cells to DNA damage-inducing agents, modified transplantation conditioning regimens are required. For this reason, it is critical to identify patients with FA as having the condition before proceeding to HSCT. Patients with FA who present with MDS or AML without an observed BM failure phase may go unrecognized until the use of standard remission induction or transplantation conditioning regimens results in excessive nonhematologic toxicity.

HSCT corrects only the hematopoietic defect, and the patient remains at risk for FA-related complications in other tissues, such as solid tumors. Moreover, some studies have suggested that HSCT in FA is associated with an increased risk of subsequent solid tumors, particularly in the setting of chronic graft-versus-host disease (GVHD). Despite these limitations, HSCT from a matched (unaffected) sibling may be considered as the initial treatment of choice for patients with FA who present with BM failure. Outcomes of unrelated donor HSCT, while historically very poor, have improved with FA-tailored conditioning regimens and when carried out at centers with expertise in FA transplants. Transplantation outcomes are better if transplantation occurs before the development of leukemia, so regular surveillance of the peripheral blood counts and BM is recommended.

Androgens (eg, oxymetholone with a starting dose of 0.5 mg/kg/day) may elevate the blood counts in a subset of patients with FA. Red blood cell counts are most often improved, although improvements in platelet and neutrophil counts also may occur. Responses may be delayed, particularly for platelets, where first responses have been reported as far as 6 months out from initiation of treatment. The neutrophil count may also respond to G-CSF. Some patients who initially respond to androgens may become refractory over time. Supportive therapy with transfusions can be considered, but in candidates for allogeneic HSCT, the use of transfusions should be minimized to prevent alloimmunization, and transfusions should never be from family members. Iron overload may develop in patients receiving chronic red blood cell transfusions.

Because of the risk of neoplasia, patients with FA should undergo regular screening for cancer. Although there is no consensus on optimal frequency of such screening evaluations, annual gynecologic examination for female patients is recommended after menarche, and regular dental care is also important, with careful examination for head and neck cancer. Surveillance with liver ultrasound at least once yearly is recommended for patients undergoing treatment with androgens.

KEY POINTS

- FA is usually an autosomal recessive and rarely X-linked recessive cause of BM failure that is due to a germ line defect in DNA repair.
- Approximately 80% of patients with FA develop signs of BM failure, but the absence of marrow failure does not rule out FA if typical physical stigmata are present; conversely, the absence of physical stigmata also does not rule out FA.
- The diagnostic test for FA is a DEB or MMC chromosome breakage study.
- Patients with FA are at risk for MDS/leukemia, and solid tumors.
- FA can present in adulthood and without classic features other than BM failure or cancer.
- HSCT is the only curative option for FA-associated hematologic manifestations.
- Chemotherapeutic agents and radiation are poorly tolerated; attenuated conditioning regimens are necessary for HSCT.
- Careful monitoring for malignancies allows early institution of treatment, with attention to minimizing exposure to chemotherapy and radiation.

Dyskeratosis congenita and the telomere biology disorders

CLINICAL CASE

A 16-year-old boy presents to his doctor with a history of skin changes, nail abnormalities, and bruising. Following referral to the hematologist, examination shows he has significant nail dystrophy and reticulate skin pigmentation around the neck. Blood counts reveal moderate pancytopenia, and the BM cellularity is found to be markedly reduced. Peripheral blood chromosomal breakage analysis following exposure to DEB is normal. Subsequent tests, however, show he has very short telomeres and a missense mutation in the *DKC1* gene, confirming a diagnosis of X-linked dyskeratosis congenita.

Clinical features

The telomere biology disorders (TBDs), or telomeropathies, encompass a spectrum of diseases, which may present in early infancy to middle adulthood with clinically significant single- or multisystem involvement. While different names have been used to describe the various presentations of the TBDs (eg, DC, Hoyeraal-Hreidarsson syndrome [HHS], and familial MDS), they all share the underlying molecular defect of abnormally short telomeres for age.

Clinical features of classic DC often appear in childhood. Historically, DC was diagnosed based on the presence of a mucocutaneous triad of abnormal skin pigmentation, nail dystrophy, and leukoplakia. The abnormal skin pigmentation and nail changes usually appear first and become more pronounced over time. The onset is usually prior to age 20 years; in many cases before the age of 10 years. BM failure develops frequently before the age of 20 years with up to 80% of patients showing signs of BM failure by the age of 30 years. However, there is considerable variation between patients with respect to age of onset and disease severity even within the same family, which can make rendering of a diagnosis based on clinical features challenging. Equally, it is not uncommon for the BM failure or an abnormality in another system to present before the more classic mucocutaneous features, and this is being recognized increasingly since the advances in the genetics of the TBDs and telomere length testing. In some cases, patients have been diagnosed with DC in the years following HSCT for AA, after development of the mucocutaneous triad at first mistaken for chronic GVHD.

Patients with telomeropathies also are at risk for pulmonary fibrosis, cirrhosis, hepatopulmonary syndrome, and hematologic and solid malignancies, particularly head and neck squamous cell carcinoma. The main causes of mortality in DC and related telomeropathies are BM failure (~60% to 70%), pulmonary disease (~10% to 15%), and malignancy (~10%).

HHS is a severe multisystem telomere biology disorder, characterized by growth retardation of prenatal onset, microcephaly, cerebellar hypoplasia, BM failure, and immunodeficiency. Revesz syndrome, which also manifests in infancy, is used to describe those patients who have bilateral exudate retinopathy along with other features of DC and HHS.

Telomere length testing is an important component in the diagnosis of DC and other TBDs as it is now available on a clinical basis from CLIA-approved laboratories. Telomere flow-FISH (fluorescent *in situ* hybridization) combines flow cytometry with fluorescence *in situ* hybridization to measure the average telomere lengths in total lymphocytes, specific lymphocyte subsets, and granulocytes. Telomere length below the first percentile for age in lymphocyte populations is generally consistent with a diagnosis of DC or a TBD. Telomere length below the first percentile for age in granulocytes is nonspecific.

With the advancement of genetics and telomere length testing, adult-onset disease due to telomere length defects is increasingly appreciated. This includes AA in the absence of the mucocutaneous triad characteristic of DC, MDS without a preceding diagnosis of AA, and rarely AML. Up

to 15% of cases of familial pulmonary fibrosis are due to mutations in telomere biology genes. A personal or family history of AA in an individual with pulmonary fibrosis is highly predictive of an underlying TBD. Similarly, a personal or family history of pulmonary fibrosis in a person with AA should prompt consideration of a TBD.

Pathophysiology

Fourteen genes have been associated with the TBDs to date. Figure 16-4 shows the different components of the telomerase and shelterin complexes as well as other factors important in telomere maintenance. Autosomal dominant, autosomal recessive, and X-linked recessive inheritance is reported. Early childhood onset and multisystem disease is most often associated with X-linked recessive mutations in *DKC1*, heterozygous de novo mutations in *TINF2*, and biallelic mutations in *RTEL1* and *TERT*. DC or adult presentation of hematologic, pulmonary or liver disease is typically associated with heterozygous mutations are *TERT*, *TERC*, *RTEL1*, or *PARN*.

Telomerase is a specialized reverse transcriptase that adds the telomeric repeat (TTAGGG) to the 3' end of the DNA strands after replication. It is composed of 2 core components: a catalytic component, encoded by *TERT*, and an integral RNA subunit, encoded by *TERC*, which includes the template for the telomeric repeat addition. Because of the semiconservative nature of DNA replication, telomerase is essential to maintain telomere length in rapidly dividing cells, such as cells of the hematopoietic system, including activated T cells and monocytes. Telomerase is also expressed in germ cells, stem cells, and their immediate progeny. Without telomerase, the telomeres shorten with each successive round of replication, and when they reach a critical length, the cells enter senescence. In cells in which telomerase is not present, telomere shortening is part of the normal process of cellular aging. BM failure in patients with very short telomeres is thought to be driven by premature loss of hematopoietic stem and progenitor cells senescence. Defects in the BM niche may also contribute.

Treatment

BM failure is the main cause of premature mortality in DC. Anecdotal reports and small retrospective case series suggest anabolic steroids (oxymetholone and danazol) can produce improvement in hematopoietic function. Approximately two-thirds of patients with DC respond to oxymetholone or danazol; in some cases, the response can last several years and involve all lineages. Patients with DC can respond to a dose as low as 0.25 mg of oxymetholone/kg/day and this can be increased, if necessary, to

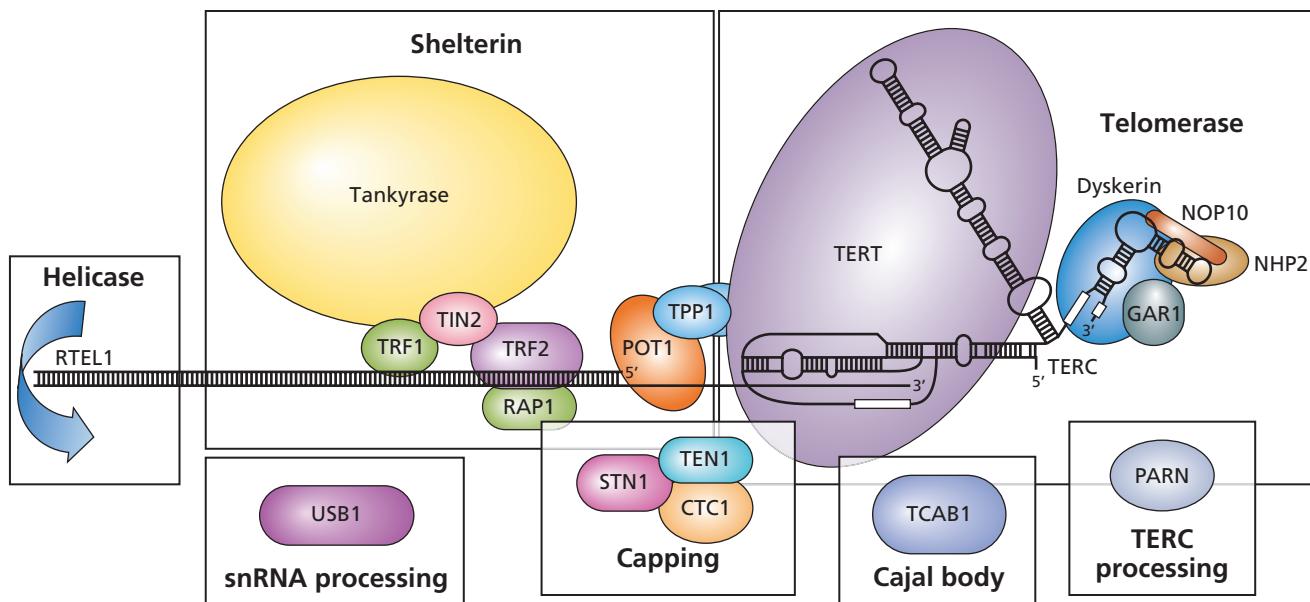


Figure 16-4 Complexes important in telomere maintenance. A schematic representation of the telomerase complex (dyskerin, GAR1, NHP2, NOP10, TERC, and TERT), the shelterin complex, and their association with different categories of dyskeratosis congenita and related diseases. The minimal active telomerase enzyme is composed of of TERT, TERC (a nontranslated RNA), and dyskerin. PARN is involved in the processing of TERC, whereas dyskerin, GAR1, NHP2, and NOP10 are believed to be important for the stability of the telomerase complex. The shelterin complex is made up of 6 proteins (TIN2, POT1, TPP1, TRF1, TRF2, and RAP1) and is important in protecting the telomere. Mutations in components of the telomerase complex, the shelterin complex and related molecules, as occurs in different subtypes of DC and related disorders, result in telomere shortening.

2 to 5 mg/kg/day. A prospective study of adults with AA and an underlying TBD demonstrated that danazol is associated with increases in telomere length in peripheral blood mononuclear cells. It is important to monitor for side effects (eg, liver toxicity). The concurrent use of androgen and G-CSF is not recommended due to reports of splenic peliosis, with or without splenic rupture, in patients with DC receiving these treatments simultaneously.

The only long-term treatment for the hematopoietic abnormalities is allogeneic HSCT. Historically, significant mortality was associated with BM transplants for patients with DC, with the conditioning regimen appearing to have an impact on patient survival. The standard myeloablative conditioning regimens are associated with frequent and severe adverse effects, such as pulmonary complications and veno-occlusive disease. The adoption of nonmyeloablative fludarabine-based protocols has allowed for successful engraftment in some patients with fewer complications and lower toxicity. The long-term survival, however, is unknown at present but the initial response is encouraging. As with FA, patients with DC need to be followed up long term for nonhematological complications, which represent the natural history of the disease and are not corrected by HSCT.

KEY POINTS

- DC is a marrow failure syndrome classically characterized by the triad of dystrophic nails, reticular skin pigmentation, and oral leukoplakia.
- Nonhematologic clinical features usually develop later in life, may be absent in young children, and may be mistaken for chronic GVHD in patients who received HSCT for AA.
- The TBDs are associated with an increased risk for MDS, AML, and squamous cell carcinomas.
- The TBDs are associated with genetic defects in telomere maintenance. Very short telomere lengths are seen in these patients.
- Numerous genes encoding factors required for normal telomere maintenance have been implicated in the TBDs to date.
- Clinical presentation can range from AA alone to severe forms, such as HHS and Revesz syndrome.
- The co-occurrence of AA with a personal or family history of pulmonary fibrosis should provoke testing for an underlying TBD.

Shwachman-Diamond syndrome

Clinical features

SDS is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency, BM dysfunction, and other somatic abnormalities. It has an estimated incidence of 1 in 77,000. While classically defined by presence of exocrine pancreatic insufficiency with neutropenia (ANC <1,500/ μ L) on at least 3 separate occasions, registry data on genetically diagnosed patients indicate that only ~50% of patients present with steatorrhea and neutropenia. Signs of pancreatic insufficiency (malabsorption, failure to thrive) are apparent early in infancy, with pancreatic function improving in a subset of patients. Additional features present in 20% to 40% of patients include skeletal system abnormalities, elevated liver enzymes, cardiac abnormalities, and eczema. Metaphyseal dysostosis is seen on radiographs with the localization and severity varying with age.

The spectrum of hematological abnormalities includes neutropenia (~60%), other cytopenias (~20% have pancytopenia), MDS, and leukemic transformation (~25%). As these complications may not develop until adulthood, it is important to continue close hematological follow-up throughout life. Isochromosome 7q and del(20q) are very frequent in SDS, but do not imply a poor prognosis by themselves, as they may be stable or decline over time. The age at which leukemia develops varies widely from 1 to greater than 40 years. The development of leukemia, often with features of MDS, usually has a poor prognosis. AML, particularly with erythroid differentiation, is the most common, and there is an unexplained preponderance of cases of leukemia in males (male:female ratio ~3:1). Advances in DNA sequence analysis have increased awareness that SDS may present as MDS or AML in young adulthood and is associated with very poor outcomes.

Exocrine pancreatic insufficiency and hematological abnormalities are also seen in Pearson syndrome, a fatal multiorgan mitochondrial disease presenting in infancy with neurological, pancreatic and BM failure, and is therefore important in differential diagnosis of very young patients. Other differential diagnoses to be excluded are cartilage hair hypoplasia syndrome and cystic fibrosis.

Pathophysiology

The majority (~90%) of patients with SDS have been found to have biallelic mutations in the gene *SBDS*. The *SBDS* protein has an important role in the joining of the 40S and 60S ribosomal subunits to form the 80S ribosome (Figure 16-5). SDS therefore can be regarded as a disorder of ribosome biogenesis, similar in some respects to DBA and del(5q) syndrome with *RPS14* haploinsuffi-

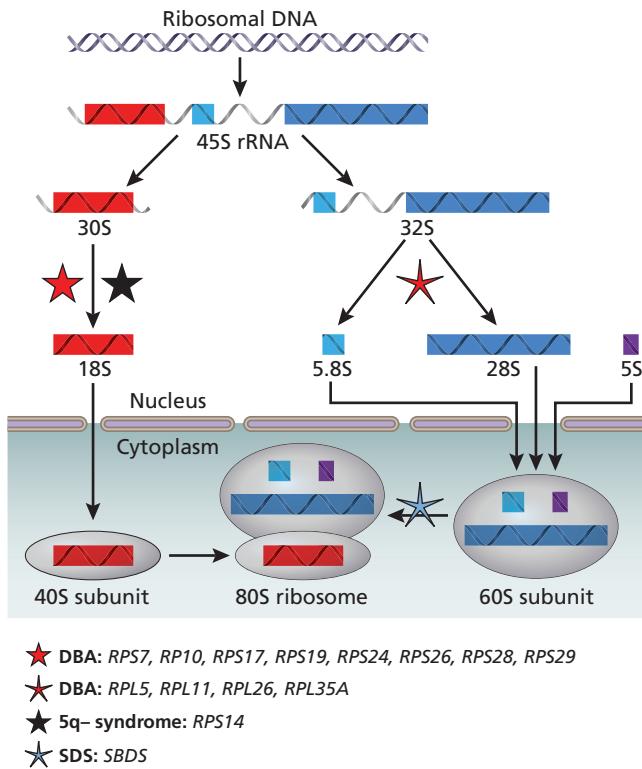


Figure 16-5 Ribosome biogenesis. Schematic showing scheme of rRNA processing in human cells and the points at which this possibly is disrupted in the different BM failure syndromes. The ribosomal RNAs (rRNAs) are transcribed by RNA polymerase I as a single precursor transcript (45S rRNA). The 45S rRNA is then processed to 18S, 5.8S, and 28S rRNAs. The 18S is a component of the 40S ribosomal subunit. The 5.8S and 28S together with 5S (synthesized independently) are components of the 60S ribosomal subunit. The 40S and 60S subunits are assembled to form the 80S ribosomes. The processing steps affected in Shwachman-Diamond syndrome (most often biallelic mutations in *SBDS*), Diamond-Blackfan anemia (most often due to heterozygous mutations in *RPS19*, *RPL5*, *RPS26*, *RPL11*, *RPL35A*, and *RPS24*) and 5q- syndrome (haploinsufficiency of *RPS14*) are indicated by the different colored stars.

ficiency. Neutrophil chemotaxis defects are also observed in SDS and may contribute to infection risk. The mechanisms underlying the development of neutropenia, BM failure, and clonal evolution to MDS/AML are poorly defined. Clonal hematopoiesis due to mutations in *TP53* is observed in a substantial number of cases and is likely an early driver in leukemogenesis. Alterations of the bone marrow niche have been proposed to contribute to genotoxic stress and clonal evolution.

Recently, rare patients with SDS or an SDS-like syndrome lacking mutations in *SBDS* were found to have mutations in *DNAJC21* or *ELF1*, which also encode factors involved in ribosome biogenesis. In addition, an analysis of patients who received HSCT for MDS uncovered germline biallelic *SBDS* mutations in several young adults (age

<40 years) who were not previously diagnosed with SDS, suggesting it may be underdiagnosed.

Treatment

The malabsorption in SDS responds to treatment with oral pancreatic enzymes. For those with neutropenia, G-CSF may produce an improvement in the neutrophil count and with responses typically observed with lower doses of G-CSF than those required for patients with severe congenital neutropenia (SCN). As in other cases of BM failure, supportive treatment with red cell and platelet transfusions and antibiotics is very important. Allogeneic HSCT is potentially curative for the hematologic manifestations of SDS. Historically, outcomes have been poor for these patients; however, these have improved with attempts to reduce regimen-related toxicity.

SDS patients with leukemia treated with conventional courses of chemotherapy usually fail to regenerate normal hematopoiesis, likely due to the constitutional defects in HSC. Therefore, for those who develop leukemia, the only approach likely to be successful is allogeneic HSCT using low-intensity conditioning regimens.

KEY POINTS

- SDS is a rare autosomal recessive disorder characterized by BM failure and exocrine pancreatic insufficiency.
- The majority of patients with SDS have biallelic mutations in the *SBDS* gene, which has an important role in ribosome biogenesis.
- Like other BM failure syndromes, patients with SDS have a high risk of developing MDS and leukemia.
- Patients with isolated neutropenia can be treated with G-CSF; those developing more global BM failure can be treated with HSCT.

Diamond-Blackfan anemia

CLINICAL CASE

A 6-month old female infant was evaluated by her pediatrician for failure to thrive. She had marked pallor and was noted to have bilateral hypoplastic thumbs. A complete blood count demonstrated normal leukocyte and platelet numbers, with severe macrocytic anemia. BM examination revealed mild hypocellularity with an M:E ratio of >20:1 and no dysplastic features. There was no increase in chromosomal breakage with DEB. Mutation testing of the patient and her parents revealed a sporadic mutation in the ribosomal protein gene *RSP19*.

Clinical features

DBA classically presents in infants by several months of age, but children up to age 2 can fall within the DBA disease spectrum. Patients have hypoproliferative, macrocytic anemia. BM examination typically reveals a profound paucity of erythroid precursors. Differential diagnosis includes FA, acute or chronic parvovirus B19 infection, and transient erythroblastopenia of childhood.

Inheritance is autosomal dominant, with variable penetrance, but many patients present without a family history, and presumed or documented sporadic mutations. At least 50% of patients have at least 1 congenital anomaly, which may involve the thumb and radius, head and face (eg, cleft palate), genitourinary tract, and heart. In addition, many patients have constitutional short stature. However, these anomalies may be subtle, and patients almost always present to medical attention for anemia.

The incidence is approximately 1 per 150,000. Both sexes are equally affected, with no ethnic predisposition. Red blood cell adenosine deaminase levels are elevated in most patients and can assist in diagnosis. Patients with DBA have a 5-fold increased risk of cancer, most markedly colon cancer, osteogenic sarcoma, acute myeloid leukemia, and female urogenital cancers.

Pathophysiology

Heterozygous germ line mutations in the *RPS19* gene, which encodes a ribosomal protein, were the initial genetic defect linked to DBA, found in approximately 25% of patients. Germline mutations in at least 19 other genes encoding ribosome-associated proteins have also been described in families with DBA who have wild-type *RPS19*. It is not clear how a defect in 1 allele of a gene encoding a ribosomal protein leads to red blood cell hypoplasia and not to other dramatic phenotypic manifestations, because ribosomes are essential for all cellular protein synthesis. Differences in spatial-temporal expression of ribosomal genes may play a role, as well as differential tissue or lineage responses to ribosomal stress.

Intact ribosomes appear to be particularly important for normal erythropoiesis; in patients with acquired MDS associated with the deletion of chromosome 5q, acquired haploinsufficiency of *RPS14* (a gene at 5q31 that encodes another ribosomal component) contributes to disease-associated anemia and provides support for the concept that erythropoiesis is uniquely sensitive to ribosomal dysfunction.

Recently, germ line mutations in the X-linked *GATA1* gene have been identified in some male patients with DBA. Patients with adenosine deaminase 2 deficiency due to biallelic mutations in *CECR1*, which can encompass a

spectrum of clinical phenotypes, may present with a pure red cell aplasia and be misdiagnosed with DBA. A homozygous mutation in the EPO gene was linked to congenital anemia not responsive to hematopoietic stem cell transplantation, but improvement with pharmacologic EPO therapy. Currently, up to 30% of patients with a clinical DBA phenotype do not have mutations in previously identified DBA genes.

Treatment

In the majority of patients with DBA (~70% to 80%), the hemoglobin level improves with corticosteroid treatment. A therapeutic trial of corticosteroids, however, is generally not initiated in infants to avoid the profound impact of corticosteroids on growth and vaccine responses; instead, patients are supported with red cell transfusions until the age of 12 months. It is vital to use the minimal dose of steroids required to support erythropoiesis to minimize adverse effects of chronic steroid use. Patients whose anemia does respond to steroids, or who require high steroid doses, may be supported with red blood cell transfusions instead. The anemia may spontaneously remit later in childhood, but up to 40% of patients remain dependent on long-term red blood cell transfusions. Careful attention to iron overload via tracking of ferritin levels and periodic liver T2^{*} magnetic resonance imaging (MRI) is critical, with timely initiation of iron chelation therapy important for DBA patients, particularly those being prepared for allogeneic transplantation and those being supported on chronic transfusions long-term. Currently, the only curative treatment of marrow failure in DBA is allogeneic HSCT, but the risks must be weighed against the benefits for each patient. The decision to move to transplantation can be challenging, since younger patients with minimal iron overload do best with transplantation, however, anticipation of a possible spontaneous remission and long-term transplantation toxicities weigh against early intervention.

KEY POINTS

- DBA typically presents in infancy with macrocytic anemia, reticulocytopenia, and marked loss of marrow erythroid precursors.
- Approximately 50% of patients with DBA have physical signs, most frequently thumb, radial and craniofacial abnormalities.
- Autosomal dominant mutations in multiple different ribosomal genes can be identified in ~65% of DBA patients; hemizygous mutations in *GATA1* are linked to some additional cases.

- Treatment options for DBA include corticosteroids, red blood cell transfusion support with iron chelation, and allogeneic HSCT.
- Spontaneous remissions may occur in a subset of patients.
- DBA patients have an increased risk of certain solid tumors and AML.

Congenital dyserythropoietic anemias

General clinical features

The congenital dyserythropoietic anemias (CDAs) are a heterogeneous group of conditions characterized by ineffective erythropoiesis and anemia, multinucleated erythroid precursors in the marrow, and excess iron even in the absence of blood transfusions. Beyond these similarities, the subtypes of CDA have differing clinical features and modes of inheritance. Two types of CDA (CDA I and the more common CDA II) are fairly well defined; CDA III is rare, and the other forms of CDA are very rare and poorly characterized. The differential diagnosis of dyserythropoiesis includes other conditions, such as hemoglobinopathies, hereditary sideroblastic anemias, *GATA1* mutations, and MDS; these should be ruled out.

CDA type I

CDA I is an autosomal recessive disorder that usually presents in childhood or adolescence. CDA is characterized by hemolytic anemia (usually moderate, with hemoglobin in the range of 9 to 10 g/dL), anisopoikilocytosis, normal or elevated reticulocyte count, macrocytosis, and high serum iron levels due to increased iron absorption. Jaundice and splenomegaly are frequent features. Some patients have skeletal anomalies. BM examination shows erythroid hyperplasia, binucleated erythroblasts, and a distinctive pattern of internuclear chromatin bridging.

Approximately 90% of CDA I cases (CDA 1a) are due to mutations in *CDAN1*, which encodes codanin 1, a protein of poorly defined function. Some of the remaining cases (CDA 1b) are due to biallelic mutations in *C15orf41*. For unclear reasons, the anemia in CDA I typically responds to recombinant interferon- α . Folate supplementation is helpful, given the chronic hemolysis. Most patients with CDA I typically do not require transfusions, and transfusions can exacerbate the tendency to iron overload. Chelation therapy may be required for iron overload.

CDA type II

CDA II is more common than CDA I (~450 patients have been collected in European registries) and patients present with anemia of variable severity most often in early childhood, although up to 40% of cases present in young adults.

Transfusion dependence is uncommon. The reticulocyte count is low, and the BM typically shows multinucleated erythroid precursors, karyorrhexis, and pseudo-Gaucher cells. The red blood cell membrane in patients with this disorder demonstrates abnormal glycosylation, apparently because of a defect in Golgi processing in erythroblasts. Abnormal migration of band 3 and band 4.5 on sodium dodecyl sulfate gels may be useful diagnostically. CDA II is an autosomal recessive disorder due to biallelic mutations in *SEC23B*, which encoded a component of the secretory COPII coat.

Like other patients with congenital dyserythropoiesis, patients with CDA II can have problems with iron overload, which is treated with phlebotomy or iron chelation. Because the osmotic fragility test is usually abnormal in CDA II, some patients are misdiagnosed as having hereditary spherocytosis and undergo splenectomy. Splenectomy may be useful in treating anemia in some patients, but results are variable.

Other CDA types

CDA III is a rare autosomal dominant disorder characterized by the presence of multinucleated erythroid precursors in the marrow (giantoblasts) in addition to mild anemia and low reticulocyte counts. Heterozygous mutations in *KIF23* have been identified in patients with CDA III. The peripheral smear shows marked anisopoikilocytosis and basophilic stippling of the red blood cells, a picture similar to β-thalassemia major. CDA IV, which is exceptionally rare, is caused by a specific dominant negative mutation (E325K) in *KLF1*, which encodes the erythroid transcription factor KLF1. Expression of KLF1-E325K results in major ultrastructural abnormalities, the persistence of embryonic and fetal hemoglobins, and the absence of some red cell membrane proteins. CDA is a feature of the autoinflammatory disease Majeed syndrome, which is discussed further in the section “Autoinflammatory diseases” in this chapter.

KEY POINTS

- CDA I is characterized by moderate hemolytic anemia, internuclear chromatin bridging, iron overload, germ line biallelic mutations in *CDAN1* or *C15orf41*, and responsiveness to interferon-α therapy.
- CDA II is the most common form of CDA and can be misdiagnosed as hereditary spherocytosis. Patients typically have multinucleated giant erythroblasts, and a low reticulocyte count. CDA II is caused by biallelic mutations in *SEC23B*.
- Several rare forms of CDA have also been genetically characterized, including those with heterozygous mutations in *KIF23* and *KLF1*.

Severe congenital neutropenia and cyclic neutropenia

CLINICAL CASE

A 4-month old male infant presented with inflamed gums and severe bacterial pneumonia, and was found to have an ANC of 20/µL, normal platelets and no anemia. The infant had no developmental abnormalities. The pneumonia was treated and weekly blood counts showed no change in the neutrophil count. Treatment with G-CSF raised the neutrophil count to 1,000/µL.

Clinical features

Congenital neutropenias include SCN, often termed Kostmann syndrome, present in infancy with fever and severe infections, resulting in early death in the absence of treatment directed at increasing the neutrophil count. The ANC is often $<0.2 \times 10^9/L$, with normal red cell and platelet counts. The BM shows maturation arrest of myelopoiesis, with abundant promyelocytes but a marked reduction in myelocytes, metamyelocytes, and neutrophils. Although the original description by Kostmann was of an autosomal recessive disorder, other congenital neutropenia subtypes (both sporadic and autosomal dominant) have been subsequently included in this category.

Congenital cyclic neutropenia (CyN) is characterized by regular cycles of severe neutropenia reaching a nadir most commonly (but not universally) every 21 days. At the nadir, patients may develop fever and mouth ulcers, and at times serious infections.

Pathophysiology

CyN families were initially identified as having autosomal dominant disease mutations in the *ELANE* gene encoding neutrophil elastase (NE). An extraordinary twist was the subsequent identification of *ELANE* mutations in many SCN families. NE is a serine protease that is synthesized predominantly at the promyelocytic stage and is likely important in neutrophil development. *ELANE* mutations lead to accumulation of a nonfunctional protein, which triggers an unfolded protein response leading to myeloid maturation arrest. Why certain mutations result in CyN vs SCN is unclear, but may relate to the short half-life of neutrophils, and homeostatic mechanisms resulting in waxing and waning neutrophil production in patients with certain CyN *ELANE* mutations, and/or modifying additional host factors. Another puzzling observation is that *ELANE*-mutated SCN patients have an increased risk of AML, but no such risk has been associated with *ELAN*-mutated CyN.

In contrast to the more common autosomal dominant *ELANE*-mutated SCN, the original family described by Kostmann had autosomal recessive SCN, subsequently linked to biallelic mutations in the *HAX1* gene. Biallelic mutations in *HAX1* account for ~10% of SCN. The HAX1 protein is a regulator of mitochondrial membrane potential and apoptosis, although it is unclear why premature death of neutrophils is specifically associated with HAX1 deficiency. Additional causes of SCN include activating mutations in the Wiscott-Aldrich syndrome (WAS) gene (in contrast to loss-of-function mutations in classic WAS with thrombocytopenia and immunodeficiency), which results in X-linked disease. Whole exome or genome sequencing of affected individuals and families continues to uncover additional mutations linked to SCN; however, in over 30% a genetic cause has yet to be identified.

Treatment

The availability of G-CSF has revolutionized the outcomes of patients with SCN and CyN. Chronic therapy increases the neutrophil count in SCN, resulting in decreased frequency of infections and increased survival. With longer survival of SCN patients since the use of G-CSF, a risk of progression to AML of 20% to 25% has been appreciated. Acquired somatic mutations in the gene that encodes the G-CSF receptor have been documented in SCN patients prior to leukemic progression. Leukemic transformation occurred in patients with congenital neutropenia prior to the availability of G-CSF, and the precise contribution of G-CSF therapy to the development of G-CSF receptor (*CSF3R*) gene mutations in SCN remains unclear. For SCN patients who become refractory to G-CSF or who develop leukemia, SCT may be appropriate and curative.

G-CSF therapy of CyN reduces the duration of neutropenic nadirs and severe infections. Of note, only a single CyN patient on prolonged G-CSF therapy has developed AML, with over 3,000 patient-years of follow-up.

KEY POINTS

- SCN presents in infancy with profound neutropenia and infection risk and can be treated effectively with G-CSF.
- CyN is characterized by approximately 21-day cycles of neutropenia, mouth ulcers, and fevers, and can be treated effectively by G-CSF.
- Both SCN and CyN are associated with autosomal dominant mutations in the neutrophil elastase (*ELANE*) gene, resulting in premature death of developing myeloid cells in the BM.
- SCN but not CyN patients have an increased risk of AML, associated with acquisition of somatic mutations in the G-CSF receptor (*CSF3R*) gene.

WHIM syndrome

WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome is a rare autosomal dominant disorder characterized by neutropenia, B-cell lymphopenia, hypogammaglobulinemia, and severe human papillomavirus (HPV) infection. Affected individuals typically present with recurrent bacterial infections in the setting of moderate neutropenia. Despite the peripheral neutropenia, the BM of affected patients is hypercellular with retention of *increased* numbers of mature neutrophils (a finding termed *myelokathexis*). Patients commonly have B-cell lymphopenia and hypogammaglobulinemia, and specific susceptibility to very extensive and progressive warts caused by HPV.

The majority of patients with WHIM syndrome have heterozygous mutations of the *CXCR4* gene. *CXCR4* is a G protein-coupled receptor for SDF1 (*CXCL12*), and SDF1/CXCR4 signaling results in neutrophil retention within the BM. The mutations of *CXCR4* in WHIM syndrome result in *enhanced* CXCR4 signaling, increasing BM neutrophil retention and causing peripheral neutropenia, and likely also result in abnormal B-cell trafficking and thus, function.

G-CSF is effective in increasing circulating neutrophil numbers but would not be expected to have any impact on the B cell abnormalities and does not impact the risk of infections in patients with WHIM. WHIM patients with significant hypogammaglobulinemia benefit from intravenous immunoglobulin therapy. Surveillance with surgical removal of dysplastic skin or mucosal HPV-related lesions is important.

Given that many of the clinical features affecting patients with WHIM are a consequence of hyperfunction of CXCR4, inhibitors of CXCR4 function, such as plerixafor, are being investigated in clinical trials and show promise for reversing neutropenia and B lymphopenia.

KEY POINTS

- WHIM syndrome is an inherited autosomal dominant syndrome characterized by HPV-related warts, hypogammaglobulinemia with recurrent infections, and BM myelokathexis resulting in neutropenia.
- WHIM syndrome is caused by heterozygous mutations in the *CXCR4* gene, resulting in functional overactivity of CXCR4/CXCL12 signaling and retention of neutrophils in the BM.
- Clinical management includes therapy with IVIg, prophylactic antibiotics, and plerixafor.
- Treatment with G-CSF is of unclear benefit.

Thrombocytopenia with absent radii

Thrombocytopenia with absent radii (TAR) is an autosomal recessive disorder characterized by hypomegakaryocytic thrombocytopenia and bilateral radial aplasia. The presence of normal thumbs distinguishes TAR from other syndromes that may manifest with blood count abnormalities and radial aplasia, such as FA. Babies with TAR often have hemorrhagic manifestations at birth (when the diagnosis usually is made), owing to the characteristic physical appearance combined with thrombocytopenia. Additional skeletal (absent ulnae, absent humeri, clinodactyly) and other somatic (microcephaly, hypertelorism, strabismus, heart defects) abnormalities may be seen in some patients.

The platelet count is usually $<50 \times 10^9/L$. The leukocyte count can be normal or raised, sometimes up to $100 \times 10^9/L$ (leukemoid reaction). BM cellularity is normal, and myeloid and erythroid lineages are normal or increased. Megakaryocytes are absent or decreased. Most patients bleed in infancy and then improve after the first year. Cow's milk allergy is common and may be associated with significant gastroenteritis and exacerbation of thrombocytopenia. The mainstay of management is prophylactic and therapeutic use of platelet transfusions. Patients with TAR have a good prognosis after infancy. There have been no reports of AA, but 5 of leukemia, including 2 adults with AML. Whether TAR is associated with an increased risk of hematologic malignancy remains unknown.

TAR is caused by compound inheritance of a low-frequency regulatory single nucleotide polymorphism and a rare microdeletion including the *RBM8A* gene. *RBM8A* encodes the Y14 protein subunit of the exon-junction complex, which is important for RNA metabolism. The mechanism by which reduced Y14 expression causes TAR is unclear but is hypothesized to be due to dysregulation of transcripts required for normal thrombocytopoiesis. Thrombopoietin levels are usually elevated and thrombopoietin receptor expression on the surface of TAR platelets is normal. Therefore, defective megakaryocytopoiesis or thrombocytopoiesis does not appear to be caused by a defect in thrombopoietin production. There is some evidence that it may be due to a lack of response to thrombopoietin in the signal transduction pathway of the thrombopoietin receptor encoded by the *MPL* gene.

- It was recently established that TAR is due to biallelic mutations in the *RBM8A* gene, which encodes a subunit of the exon-junction complex.

Congenital amegakaryocytic thrombocytopenia

CAMT is a rare autosomal recessive disorder characterized by absent or greatly diminished megakaryocytes. Patients typically present shortly after birth with symptomatic thrombocytopenia and progress to AA, in some cases after a period of improved platelet counts. CAMT is not associated with specific congenital anomalies; however, patients with CAMT and common somatic malformations have been reported. There are also case reports of patients with CAMT developing MDS and leukemia.

CAMT is caused by biallelic mutations in *MPL*, which encodes the thrombopoietin (TPO) receptor. Development of AA in patients with CAMT is consistent with findings that TPO signaling plays an important role in the maintenance and expansion of HSCs and multipotent progenitors. TPO levels are typically high in CAMT.

Supportive care consists largely of platelet transfusions. Antifibrinolytic agents may be useful to help treat bleeding. Patients who progress to aplasia can be cured with HSCT. The platelet count is not responsive to TPO.

KEY POINTS

- CAMT is caused by biallelic mutations in the *MPL* gene encoding the TPO receptor.
- CAMT presents in the neonatal period with bruising or bleeding and severe thrombocytopenia; pancytopenia may develop in later childhood.
- CAMT may be treated with HSCT.

GATA2 deficiency and similar clinical syndromes

Germline heterozygous mutations in the *GATA2* gene, which encodes a zinc finger transcription factor that is required for hematopoiesis and lymphatic development, have been linked to 4 previously described overlapping clinical syndromes, specifically monocytopenia and mycobacterial infection "MonoMAC" syndrome; dendritic cell, monocyte, B and NK cell lymphoid deficiency syndrome; primary lymphedema with myelodysplasia progressing to AML (also termed Emberger syndrome); and a subset of familial MDS. These entities are now designated GATA2 deficiency and represent the same underlying disorder with variable penetrance and severity. Inheritance is autosomal dominant; however, clinical manifestations even within the same family can be

KEY POINTS

- TAR is characterized by isolated thrombocytopenia and bilateral radial aplasia.
- The mainstay of management is platelet transfusions, and patients usually have a very good prognosis.

extremely variable. The *GATA2* mutations result in haploinsufficiency due to loss of expression or protein function, with mutations found in both zinc finger and regulatory regions.

Patients generally present in adolescence or early adulthood, most commonly with viral or nontuberculous mycobacterial infections and/or with cytopenias, often initially diagnosed as AA or MDS. Specific features suggesting *GATA2* deficiency include reduced peripheral blood monocytes, B cells and NK cells, and marrow hypocellularity with dysplastic megakaryocytes and absent B cell hematogones. Monosomy 7 and trisomy 8 are the most frequent cytogenetic abnormalities, and their presence often portends rapid progression to high-risk MDS and AML. *GATA2* mutations are the most frequent associated mutations in pediatric MDS, and the lifetime risk of MDS/AML in *GATA2* deficiency is 90%. Additional clinical findings can include pulmonary alveolar proteinosis, recurrent upper respiratory tract infections, warts, panniculitis, lymphedema, thrombosis, and hearing loss.

In addition to monitoring for infections and other non-hematological disease complications, serial blood count and annual BM evaluations are recommended. There are no specific treatments other than allogeneic HSCT. It is important to screen potential family donors for the presence of *GATA2* mutations or subtle peripheral blood phenotypes before transplantation, given variable clinical penetrance in many families. Development of chromosomal abnormalities, MDS, or AML is indication for prompt transplantation.

Gain-of-function heterozygous mutations in *SAMD9L*, a tumor suppressor located on chromosome 7q, have been recently linked to families with cerebellar dysfunction and a BM failure syndrome with some characteristics overlapping *GATA2* deficiency, including cytopenias, B/NK cell immunodeficiency, and progression to MDS with -7/del(7q) cytogenetic abnormalities. In addition, gain of function mutations in the paralog *SAMD9*, also located on 7q, result in MIRAGE syndrome, which consists of myelodysplasia, infections, restriction of growth, adrenal hypoplasia, genital abnormalities, and enteropathy. Interestingly, in both syndromes there is preferential loss of the copy of chromosome 7 that contains the mutant *SAMD9* or *SAMD9L* allele, or additional loss-of-function mutations of that allele, thought to overcome the growth-restrictive effects of the mutant proteins, but potentially resulting in MDS. Thus, screening for germline mutations in *SAMD9* and *SAMD9L* in the context of -7/del(7q) should be carried out on DNA from a nonhematopoietic source.

KEY POINTS

- *GATA2* deficiency results from heterozygous inherited loss-of-function mutations in the *GATA2* gene, a master regulator of hematopoiesis.
- *GATA2* deficiency patients can present with immunodeficiencies resulting in severe viral and atypical mycobacterial infections, cytopenias, MDS/AML, and/or lymphedema.
- *GATA2* deficiency may be treated with HSCT.

Acquired neutropenia

Neonatal alloimmune neutropenia

Physiologic and acquired neutropenia in premature infants, including neutropenia as a result of idiopathic or immune causes, is much more common than inherited or congenital neutropenia. In alloimmune neonatal neutropenia, a transplacental transfer of maternal IgG is directed against paternal antigens expressed on neonatal neutrophils. This is analogous to neonatal erythroblastosis secondary to Rh incompatibility. Rarely, the maternal antibody is secondary to autoimmune neutropenia in the mother. The incidence has been estimated at 2 per 1,000 live births. The BM usually shows normal cellularity with a late myeloid arrest. Maternal alloimmunization probably occurs during the first trimester of pregnancy. Neutrophil-specific antibodies to HNA-1a, HNA1b, and HNA-2a can be detected in more than half of cases. These children may develop omphalitis or skin infections; however, they are also at risk of severe, life-threatening infections. Aggressive antibiotic therapy must be given for documented infection, and recombinant G-CSF should be considered, although the response is variable and unpredictable. With supportive care, the neutropenia usually spontaneously resolves within 3 to 28 weeks (average of 11 weeks). Overall, the prognosis for infants with alloimmune neutropenia syndrome is favorable.

Primary autoimmune neutropenia

Primary autoimmune neutropenia typically occurs in children between the ages of 5 and 15 months but can be present from 1 month through adulthood. The ANC is typically between 500 and 1,000/mL. Serious infections are infrequent and may reflect the ability of patients to increase their neutrophil counts during acute illnesses. BM examination rarely is indicated. When performed, it reveals minimal abnormalities or only a deficit of mature neutrophils. Spontaneous remission occurs in most patients, usually within 24 months of diagnosis. The pathogenesis is

thought to be immune-mediated neutrophil clearance. Indeed, in the great majority of published cases, antibodies to neutrophil antigens can be detected, although multiple blood samples may need to be analyzed. Antibodies directed against FC γ RIII or CD11b/CD18 (also termed the type 3 complement receptor) are the most common antibodies detected. Many patients remain free of infections, and no specific therapy is required. Hence, they represent a subset of patients diagnosed with chronic benign neutropenia of infancy and childhood. For patients with recurrent infections, prophylactic antibiotics or intermittent treatment with G-CSF may be indicated. Although rarely needed, high-dose IVIg or corticosteroid therapy has been reported to be effective.

Secondary autoimmune neutropenia

Neutropenia occasionally is associated with autoimmune disease, most commonly rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjögren syndrome. Moreover, there is a strong association of neutropenia with large granular lymphocytic leukemia often in association with RA. In SLE, neutropenia occurs in ~50% of patients. The neutropenia is generally mild, has little impact on disease, and requires no specific treatment. The pathogenesis of neutropenia in SLE is thought to be related to accelerated apoptosis of mature neutrophils. Although neutrophil antibodies have been implicated, the clinical utility of measuring antineutrophil antibodies in SLE is questionable. As with SLE, the differential diagnosis for neutropenia in RA is wide, and drug-induced neutropenia must be considered. Felty syndrome is the triad of unexplained neutropenia, longstanding RA, and variable splenomegaly. There is an increased risk of infections in these patients. Treatment usually is directed at the underlying RA.

Nonimmune chronic idiopathic neutropenia

In a subset of patients with chronic neutropenia, there is no evidence of immune-mediated disease. The diagnosis of nonimmune chronic idiopathic neutropenia in adults (NI-CINA) is based on the presence of chronic acquired neutropenia in the absence of underlying autoimmune disease, cytogenetic abnormality, antineutrophil antibodies, or other obvious explanation for neutropenia. In addition to neutropenia, lymphopenia, moncytopenia, anemia, and thrombocytopenia occasionally are seen. BM findings are highly variable, with both hyperplastic and hypoplastic BM cellularity reported. The pathogenesis of NI-CINA is poorly understood, although it has been suggested that chronic low-grade inflammation may contribute. It is most common in young and middle-aged women. Fortunately, the

clinical course usually is benign, infections are infrequent, and specific treatment is not required.

Drug-induced neutropenia

CLINICAL CASE



A 50-year-old teacher with a history of ulcerative colitis presents to the emergency room with fever, chills, and sore throat for 24 hours. On examination, the temperature is 39.6°C, blood pressure 90/60, pulse 105, and respiratory rate 28. The patient is confused and reports that her throat is very sore. The abdomen is slightly tender and bowel sounds are absent. On the basis of the patient's presentation, therapeutic measures for septic shock are initiated. A complete blood count reveals a white blood cell count of $1.5 \times 10^9/L$ with an ANC of 0. On questioning the patient's husband, you learn that she had been in her usual state of health until a few days ago. She has had long-standing complaints of chronic diarrhea with intermittent blood and mucus in the stool. Her only medication is sulfasalazine begun about 3 months ago.

Non-chemotherapy drug-induced neutropenia and agranulocytosis (absence of neutrophils in the blood) are serious medical problems, with an estimated incidence of 2 to 15.4 cases per million and a case fatality rate of approximately 5%. Although certain medications carry a higher risk of neutropenia (Table 16-4), it is wise to consider most medications as potential offenders, thus emphasizing the need for a careful drug history in all patients who present with acquired neutropenia. A systematic review of the literature in 2007 identified 10 drugs that accounted for ~50% of cases of definite or probable reports of drug-induced agranulocytosis: carbimazole, clozapine, dapsone, dipyrrone, methimazole, penicillin G, procainamide, propylthiouracil, rituximab, sulfasalazine, and ticlopidine. Prolonged use of vancomycin is also associated with neutropenia. Agranulocytosis has been associated with both cocaine and heroin use. In reported cases, it was caused by the adulteration of the drug with levamisole, a drug used in veterinary medicine that is known to be associated with agranulocytosis.

In most cases, agranulocytosis presents within 6 months, and usually within 3 months, after starting the offending drug. The clinical presentation is often less dramatic than in the case described above, but patients often have fever and pharyngitis as their first symptoms. Sepsis or pneumonia may occur in 10% to 30% of patients. Usually the prognosis is good because neutrophil counts recover within approximately 1 week if the offending medication

Table 16-4 Selected drugs associated with neutropenia

Anti-inflammatory agents	Antimicrobial agents
Aminopyrine*	Ampicillin*
Diclofenac*	Cefotazime*
Diflunisal*	Cefuroxime*
Dipyrrone*	Flucytosine*
Ibuprofen*	Fusidic acid*
Gold salts	Imipenem-cilastatin*
Penicillamine	Nafcillin*
Phenylbutazone	Oxacillin*
Sulfasalazine	Quinine*
Cardiovascular agents	Ticarcillin*
Clopidogrel*	Chloramphenicol
Disopyramide*	Sulfonamides
Methyldopa*	Amodiaquine
Procainamide*	Dapsone
Quinidine*	Terbinafine
Spironolactone*	Vancomycin
Dipyridamole	Antithyroid agents
Captopril	Propylthiouracil *
Ticlopidine	Carbamazole
Anticonvulsants	Methimazole
Phenytoin*	Other agents
Carbamazepine	Amygdalin*
Psychotropic agents	Calcium dobesilate*
Chlorpromazine*	Infliximab*
Clozapine*	Levamisole*
Fluoxetine*	Metoclopramide*
Mianserin	Mebhydrolin*
Hypoglycemic agents	Rituximab
Chlorpropamide	Ranitidine
Tolbutamide	Famotidine
Glyburide*	Metiamide

*Level I evidence based on Andersohn F et al. *Ann Intern Med.* 2007;146:657–665.

is withdrawn. The disease mechanism is often unclear, although immune-mediated destruction is most often proposed. In some well-studied cases, the offending drug serves as a hapten in association with an endogenous protein, probably an antigen expressed on the neutrophil surface. The immune response to this complex results in neutrophil destruction, severe neutropenia, and susceptibility to infection. Other drugs may impair production of neutrophils by a direct toxic effect on myeloid precursors.

Drug-induced agranulocytosis is difficult to anticipate. Risk increases with age. Serial blood counts are now recommended for patients on some drugs (eg, sulfasalazine and clozapine) because of the relatively high frequency of drug-induced neutropenia associated with these agents. Practices are not standardized, and the benefit of frequent blood counts is not established.

Management includes prompt withdrawal of all potentially offending drugs and administration of broad-spectrum antibiotics, usually with inpatient management. The mean time to recovery is ~10 days, but the duration of neutropenia is highly variable. Therapy with hematopoietic growth factors, particularly G-CSF, is controversial. A number of nonrandomized trials have reported a shortened duration of neutropenia, less antibiotic use, and reduced hospital stay with the use of G-CSF. However, the only published prospective, randomized trial, which included just 24 patients, did not demonstrate a benefit of G-CSF administration, which was at a lower dose of 100 to 250 µg/day. BM examination usually is not necessary in cases with otherwise-normal hemoglobin, platelet count, and red blood cell morphology. The time to hematologic recovery may be proportional to the severity of the marrow defect; that is, if no cells at the myelocyte stage are seen on an aspirate sample, it probably will be several days before recovery occurs. A neutrophil count less than $0.1 \times 10^9/L$ and the presence of sepsis or severe infection are associated with delayed neutrophil recovery and increased mortality. Given these risks, G-CSF at a dose of 5 µg/day is recommended for these patients.

KEY POINTS

- Transient neutropenia is commonly seen in infants and children and may be due to infection, auto- or alloimmune mechanisms, unidentified causes (ie, idiopathic) or, less commonly, genetic disorders of granulopoiesis.
- The genetic basis for many congenital neutropenia syndromes has been identified and genetic testing is becoming an important diagnostic tool in the evaluation of patients with chronic neutropenia.
- Neutropenia in adults is frequently due to drugs, both as a predictable response to myelotoxic agents and as an idiosyncratic reaction to almost any drug. Less commonly, neutropenia in adults is due to infection, acquired hematopoietic disease, autoimmune disorder, or a clonal proliferation of large granular lymphocytes.

Inherited disorders of neutrophil function

CLINICAL CASE

A 2-year-old boy with consanguineous parents has had recurrent furuncles and deep abscesses since the first few months of life. On examination, there is no active infection, but there are scars from drainage of previous abscesses. CBC shows a hematocrit of 32%, WBC is $12 \times 10^9/L$, and the platelet count is $400 \times 10^9/L$. The differential count is normal, and the morphology of the leukocytes is normal. The IgG level is increased; the levels of IgM and IgA are normal. The patient's neutrophils lacked CD18 expression by flow cytometry and he was diagnosed with leukocyte adhesion deficiency type 1.

Because recurrent fevers, otitis media, and sinopulmonary infections are common in young children, it may be difficult to assess when a child has had "too many" infections despite a normal or elevated neutrophil count and requires a careful workup. Certain circumstances, however, should raise concern for an underlying neutrophil function disorder, and may merit further evaluation. These include the following: (1) severe systemic bacterial infections (eg, sepsis, osteomyelitis, meningitis); (2) infections at unusual sites (eg, hepatic or brain abscess); (3) recurrent bacterial infections (eg, pneumonia, sinusitis, severe or recurrent *Staph aureus* cellulitis, lymphadenitis, draining otitis media); (4) infections caused by unusual pathogens (eg, *Aspergillus* pneumonia, disseminated candidiasis, *Serratia marcescens*, *Nocardia* species, *Burkholderia cepacia*); and (5) chronic gingivitis or recurrent aphthous ulcers. In the previous clinical case, the history of recurrent abscesses in the setting of a normal ANC would merit further evaluation for a neutrophil function disorder. Several disorders with abnormalities of neutrophil function are described in the following sections.

Chronic granulomatous disease

CGD is a primary inherited immunodeficiency syndrome usually diagnosed in early childhood and linked to mutations in genes encoding protein components of the NADPH oxidase system. In CGD, neutrophils and monocytes are unable to generate the respiratory burst that generates superoxide, the precursor to hydrogen peroxide and other reactive oxygen derivatives with microbial activity. The disorder is characterized by recurrent bacterial and fungal infections affecting the skin, lungs, and bones with the development of granulomatous inflammatory responses in lymph nodes, gut, and other tissues.

The mutations responsible for CGD can be inherited in either an X-linked or autosomal recessive manner. About two-thirds of CGD cases are due to mutations affecting the X-linked gene CYBB, which encodes the gp91phox component of the membrane cytochrome *b* protein complex. The other cases involve mutations of autosomal genes encoding proteins in the oxidase complex. The incidence is approximately 1 in 200,000 live births in the United States.

The diagnosis of CGD is established by a typical clinical history and laboratory testing demonstrating an abnormal neutrophil oxidative burst by histochemistry (nitro-tetrazolium blue test) or flow cytometry (dihydrorhodamine assay). Genetic testing for both X-linked and autosomal recessive CGD is available.

Treatment of CGD consists of prophylactic antibiotics, antifungal agents, and the prompt administration of antibiotics, antifungals, and even neutrophil transfusions for specific infections. Chronic treatment with interferon- γ reduces the incidence of bacterial and fungal infections by ~70%. HSCT, although curative, generally is reserved for patients in whom the clinical course or specific mutation portends a poor outcome and is a high-risk procedure in CGD patients.

Leukocyte adhesion deficiency (LAD)

Leukocyte adhesion deficiencies (LAD) are very rare autosomal recessive disorders manifested by delayed wound healing, recurrent bacterial infections, and neutrophilia. There are 3 distinct types of LAD, all associated with impaired neutrophil chemotaxis and emigration from the blood to sites of infection. Mutations in β_2 -integrin (CD18) (type I), genes necessary for generation of selectin ligands (type II), or other genes impacting on integrin function (type III), have been implicated. In addition to lack of neutrophil function, patients with LADIII also have a bleeding diathesis due to a defect in integrin function on platelets. Definitive treatment of LAD requires allogeneic HSCT, with a recent study reporting a 5-year survival of 75%.

Myeloperoxidase (MPO) deficiency

Myeloperoxidase (MPO) deficiency is the most common disorder of phagocytes, with 1 in 4,000 individuals having a complete deficiency of MPO. It is inherited in an autosomal recessive fashion and is due to mutations of the MPO gene. MPO is a primary granule enzyme that catalyzes the conversion of H_2O_2 to hypochlorous acid and other toxic intermediates that greatly enhance polymorphonuclear neutrophil microbial activity. The diagnosis can be made with histochemical assays or flow cytometry for MPO in neutrophils. Of note, most patients (95%)

with MPO deficiency are asymptomatic. An increase in mucocutaneous infections with *Candida* strains has been reported, particularly in patients with concurrent diabetes mellitus. There is no specific treatment.

Hyperimmunoglobulin E syndrome (HIES)

Hyperimmunoglobulin E syndrome (HIES; previously known as Job's syndrome) is characterized by defective neutrophil chemotaxis, defective T-helper function, mild neutropenia, recurrent *Staphylococcus aureus* or *Candida* infections of the skin, sinuses, or lungs, and elevated serum IgE levels. Patients with HIES often present with severe eczema in the first few weeks of life. Autosomal dominant HIES (60% to 70% of cases) is due to heterozygous mutations in *STAT3*. Additional nonimmunologic features, such as characteristic facies, retained primary teeth, recurrent fractures, and vascular abnormalities, may be present.

Autosomal recessive HIES is most commonly due to mutations in *DOCK8* and, more rarely, *TYK2* and *PGM3*. Patients with *DOCK8* mutations lack the nonimmunologic features of *STAT3*-HIES and instead are characterized by a high incidence of atopic conditions in addition to eczema and recurrent cutaneous viral infections with herpes simplex, human papilloma, and molluscum contagiosum viruses. They also are at markedly high risk of human papillomavirus-associated squamous cell carcinomas, and Epstein-Barr virus (EBV)-associated Burkitt lymphoma and diffuse large B-cell lymphoma.

The cornerstone of treatment for HIES is antibacterial, antifungal and, for *DOCK8*-HIES, antiviral prophylaxis. HSCT is curative for *DOCK8*-HIES and, given the severity of the disease and associated mortality, should be considered early.

Chédiak-Higashi syndrome (CHS)

Chédiak-Higashi syndrome (CHS) is a rare autosomal recessive syndrome linked to mutations in the *LYST* gene and characterized by severe immunodeficiency, mild neutropenia, functional neutrophil defects, partial albinism, mild bleeding diathesis, and neurologic defects. The pathognomonic feature of CHS is the presence of giant inclusion bodies in virtually all granulated cells, particularly neutrophils (Figure 16-2). The majority of patients progress to an accelerated phase characterized by a non-clonal lymphohistiocytic infiltration of multiple organs. The loss of LYST protein disrupts vesicular trafficking, leading to hypopigmentation and abnormal granule and lysozyme formation impairing multiple functions of immune cells and platelets. Treatment of CHS and other related vesicular transport syndromes is largely supportive; or in severe cases, allogeneic HSCT.

KEY POINT

- Genetic disorders affecting neutrophil function are rare causes of recurrent infections, unexplained fever, and inflammation in children with normal or high neutrophil counts and may result from abnormalities in neutrophil adhesion and tissue entry (LAD), chemotaxis (HIES), or killing of microorganisms (CGD).

Autoinflammatory diseases

Autoinflammatory diseases, also called periodic fever syndromes, are a group of rare genetic disorders characterized by recurrent episodes of unprovoked inflammation in the absence of infection. The most common and prototypical autoinflammatory disease is familial Mediterranean fever (FMF). FMF usually presents in early childhood and is characterized by sporadic paroxysmal attacks of fever, serosal inflammation (such as peritonitis), and neutrophilia. These attacks generally last 1 to 3 days and then resolve spontaneously. FMF is inherited as an autosomal recessive disorder and mainly occurs in populations from the Mediterranean basin, with an incidence of 1 in 200 to 1 in 1,000. Mutations in the *MEFV* gene, which encodes the protein, pyrin, appear to cause dysregulation of inflammation control that leads to unpredictable episodes of neutrophil overactivity and tissue infiltration. Because the chronic, recurrent inflammatory attacks also cause persistent elevations of serum amyloid A protein, patients with FMF are at high risk of developing complications of amyloid A amyloidosis, especially in the kidneys. The diagnosis of FMF usually is made based on clinical criteria, including unexplained episodes that persist over many months to years in the absence of other etiologies of inflammation. Most of the common *MEFV* mutations are well characterized. Thus, the diagnosis can be confirmed genetically. Colchicine prevents clinical attacks and tissue amyloid deposition in most patients with FMF. Rare patients with severe refractory disease have undergone successful HSCT.

FMF must be distinguished from other autoinflammatory diseases, of which there is an ever-increasing number. Hyper-IgD syndrome (also known as mevalonate kinase deficiency) is another rare autosomal recessive autoinflammatory disease and is associated with mutations in the mevalonate kinase gene, *MVK*. The TNF receptor-associated periodic syndrome (TRAPS; previously known as familial Hibernian fever) is an autosomal dominant disorder associated with mutations in the gene-encoding TNF receptor 1, *TNFRSF1A*. Cryopyrin-associated periodic syndromes are a group of autosomal dominant inherited disorders that are caused by mutations of a pyrin-like

protein called NALP3, encoded by the *CIAS1* gene. The type of *CIAS1* mutation determines the clinical severity. Familial cold autoinflammatory syndrome is the most severe form of cryopyrin-associated periodic syndrome, followed by Muckle-Wells syndrome. Although neutrophils are not the primary mediators of pathogenesis in these non-FMF disorders, they share many clinical features with FMF and should be considered in the differential diagnosis of unexplained recurrent fever with noninfectious autoinflammation.

Mutations in *PSTPIP1* cause 2 distinct, albeit overlapping, autosomal dominant autoinflammatory syndromes—pyogenic arthritis, pyoderma gangrenosum and acne (PAPA), and *PSTPIP1*-associated myeloid-related proteinemia inflammatory (PAMI) syndromes. *PSTPIP1* encodes proline-serine-threonine phosphatase-interacting protein 1, which binds pyrin. Specific mutations in *PSTPIP1* cause PAMI syndrome, which is characterized by chronic neutropenia, rather than neutrophilia, and hyperzincemia. Thus, PAMI syndrome should be considered in the differential of patients with unexplained chronic neutropenia and arthritis and can be screened for by measuring a serum zinc level.

Majeed syndrome is a rare autoinflammatory syndrome characterized by sterile, chronic recurrent multifocal osteomyelitis, with pain and swelling around joints, recurrent febrile episodes, and CDA. Inflammatory dermatoses and hepatosplenomegaly may be present. Patients present during the first 2 years of life. The anemia is hypochromic and microcytic and may be mild or transfusion dependent. Majeed syndrome is an autosomal recessive disorder caused by mutations in *LPIN2*, which encodes lipin-2, a phosphatidic acid phosphatase. The role of lipin-2 in the control of chronic inflammation remains to be elucidated. Nonsteroidal anti-inflammatory drugs are moderately helpful. Clinical improvement was reported in 2 brothers with Majeed syndrome with interleukin-1 inhibition.

KEY POINTS

- Recurrent inflammation mimicking infection is a hallmark of the autoinflammatory syndromes such as FMF.
- PAMI syndrome should be considered in individuals with chronic neutropenia accompanied by arthritis and is associated with elevated serum zinc levels.
- Majeed syndrome should be considered in children with CDA accompanied by periodic fever and recurrent multifocal osteomyelitis.

Acquired and inherited disorders of histiocytes and dendritic cells

The histiocytoses represent a broad spectrum of disorders characterized by infiltration and accumulation of dendritic cells, macrophages, or monocyte-derived cells in a wide range of tissues and organs. They may present from mild, self-limited disease to life-threatening conditions. In 2016, the Histiocytic Society proposed a revised classification to include 5 major groups: (1) Langerhans-related (L group), (2) cutaneous and mucocutaneous (C group), (3) malignant histiocytoses (M group), (4) Rosai-Dorfman disease (R group), and (5) hemophagocytic lymphohistiocytosis and macrophage activation syndrome (H group). Representatives of each of these groups are discussed in this section.

Hemophagocytic lymphohistiocytosis and macrophage activation syndrome

CLINICAL CASE

A 9-month-old girl is admitted to the hospital after presenting with fever of 40.5°C, sore throat, and lethargy. Over the course of the next 48 hours, the child continues to have high fevers despite broad-spectrum antibiotics and develops progressive splenomegaly and pancytopenia. Laboratory data are also notable for a markedly elevated ferritin level of 24,000 ng/mL (normal 476 ng/mL) and hypofibrinogenemia. A BM biopsy reveals marked histiocyte hyperplasia with hemophagocytosis. She begins treatment with dexamethasone and etoposide. Mutational testing reveals the presence of a homozygous mutation in the *PRF1* gene.

Hemophagocytosis is the histologic finding of activated macrophages engulfing leukocytes, erythrocytes, platelets, and their precursor cells. Hemophagocytosis may be observed in a variety of conditions, including hemolytic anemias, infections, and malignancies. It also is a principal feature of hemophagocytic lymphohistiocytosis (HLH), a clinical syndrome characterized by fever, pancytopenia, and splenomegaly that results from the abnormal activation and proliferation of cytotoxic T-lymphocytes and tissue macrophages (Figure 16-6). The major pathophysiological abnormality in HLH is the high production of inflammatory cytokines with abnormal T-cell activation. Severe impairment in NK cell activity and cytotoxic T-cell function are also characteristic of the disease.

HLH most often occurs in infants and toddlers but may also be observed in children and adults of all ages. It may occur either as an inherited or acquired disorder

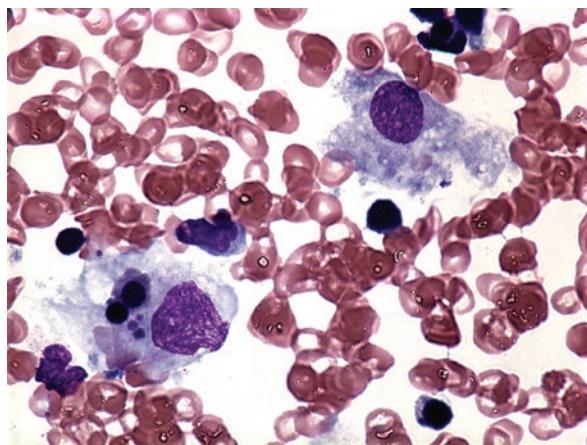


Figure 16-6 Hemophagocytic lymphohistiocytosis. BM aspirate demonstrating phagocytic histiocytes with ingested platelets and RBC precursors. From the ASH Image Bank (image 3502).

(Table 16-5). Familial hemophagocytic lymphohistiocytosis (FHL) classically presents in infancy and early childhood with an estimated incidence of approximately 1 in 50,000. FHL is caused by autosomal recessive mutations in genes that encode critical components of the granule exocytosis pathway, which enables NK cells and cytotoxic T-lymphocytes to induce apoptosis in target cells. Disease manifestations in familial forms of HLH are frequently triggered by an infection. A genetic diagnosis can be established in most infants presenting with HLH. There are 5 FHL subtypes. FHL-2 is caused by mutations in *PRF1*, which encodes perforin. Perforin is the major component of the cytolytic granules and forms the pore at the synapse between the effector lymphocyte and the target cells through which the cytolytic contents are released to initiate cell death. FHL-3, FHL-4, and FHL-5 are caused by mutations in the Munc13-4 (*UNC13D*), syntaxin 11 (*STX11*), and syntaxin binding protein 2 (*STXBP2*) genes, respectively. The mutation in FHL-1 is known to map to 9q21.3-q22, but the gene remains unknown. In addition to FHL, a clinical HLH syndrome can also occur in the context of various other inherited immune deficiency syndromes, including CHS (*LYST*), Griscelli syndrome type II (*RAB27A*), X-linked proliferative disease (XLP, caused by mutations in *SH2D1A*), X-linked inhibitor of apoptosis (XIAP) deficiency syndrome (*XIAP*), Hermansky-Pudlak type II (*AP3B1*), and GATA2 deficiency. HLH may be the presenting manifestation of a primary immune deficiency.

Acquired HLH syndrome, also known as reactive hemophagocytic syndrome or secondary HLH (sHLH), can affect adults, especially, and children and usually is associ-

Table 16-5 Hereditary and acquired causes of HLH

Primary HLH
Familial HLH
Chédiak-Higashi syndrome
Griscelli syndrome
XLP
XIAP deficiency syndrome
Hermansky-Pudlak syndrome type II
GATA2 deficiency
Secondary HLH
Infections
Herpesvirus infection, particularly EBV, CMV, HHV-8, HSV
HIV
Parvovirus, adenovirus, hepatitis virus
Bacterial, rickettsial, fungal, and spirochete-associated infections
Malignancy
AML, MDS, lymphomas, multiple myeloma
Metastatic carcinoma, metastatic melanoma
Autoimmune diseases (macrophage activation syndrome)
Other immunodeficiency states
Posttransplant
Cytotoxic or immunosuppressive therapy
Postsplenectomy

CMV, cytomegalovirus; HHV-8, human herpesvirus 8; HSV, herpes simplex virus.

ated with an underlying infection (especially viral), hematologic (particularly lymphoma) or (less commonly) non-hematologic malignancy, autoimmune, or rheumatologic disorders, AIDS (with or without opportunistic infections), posttransplantation immunosuppression, or other immunocompromised state. The pathophysiology of sHLH appears to be similar to that of FHL, except that the underlying predisposing disorder is primarily responsible for the dysregulation of T-cells and NK cells that leads to histiocyte activation.

The clinical presentation, laboratory features, and histopathology of inherited and acquired HLH are similar. HLH should be considered in the differential diagnosis in patients who develop sepsis or multiorgan dysfunction in the setting of fever, unexplained progressive pancytopenia, and hepatosplenomegaly. Lymphadenopathy, rash, and liver disease also may be present. Neurologic symptoms due to central nervous system involvement are present in one-third of patients. Laboratory findings include elevated ferritin, liver enzymes, triglycerides with low fibrinogen, and soluble interleukin-2 receptor alpha (sCD25 or sIL-

2R), decreased NK cell cytotoxic activity, and coagulation abnormalities. A BM biopsy is helpful to identify histiocytic hyperplasia and hemophagocytosis of nucleated cells, and to exclude malignancy or to identify an infectious trigger for HLH. Hemophagocytosis, however, is highly variable and may not be observed early in the clinical course. If hemophagocytic activity is not proven at the time of presentation, further search for hemophagocytic activity is encouraged but not mandatory for diagnosis if other markers are consistent with the disease. If the BM specimen is not conclusive, material may be obtained from other organs.

Diagnostic criteria for HLH have been established by the Histiocyte Society (Table 16-6). At least 5 of 8 clinical criteria or the presence of either familial disease or one of the known genetic abnormalities is required for diagnosis of HLH.

Although less severe sHLH may resolve after treatment of the underlying condition or with a short course of immunosuppression, untreated FHL is uniformly fatal within 1 to 2 months. Given it generally takes time to differentiate sHLH from FHL, early intervention is advocated for critically ill or deteriorating patients. The HLH-94 protocol of the Histiocyte Society is the current standard of care. It consists of an initial 8 weeks of dexamethasone

and etoposide followed by a continuation phase for those patients with familial, relapsing, or severe and persistent disease which consists of cyclosporine A with pulses of etoposide and dexamethasone until an acceptable donor is available for HSCT. Intrathecal therapy with methotrexate is administered in a subset of individuals with evidence of central nervous system (CNS) involvement. If a secondary “trigger” is identified, specific therapy against a specific infection, autoimmune disease, or malignancy is appropriate along with immune suppression. Results of HLH-94 demonstrated a 3-year survival rate of 51%, with comparable outcomes for FHL and sporadic HLH. Modifications to this protocol were tested in HLH-2004, however, this was found to not improve outcomes significantly and, therefore, HLH-94 remains the standard of care. A single-center retrospective analysis of FHL patients treated with ATG, prednisone, maintenance cyclosporine, and intrathecal methotrexate and corticosteroids reported 82% short-term complete response rates in treatment-naïve patients.

Macrophage activation syndrome (MAS) is considered to be a variation of sHLH and occurs in individuals with autoimmune disorders. The disorder is most frequently seen in systemic juvenile idiopathic arthritis (SJIA) but can also be observed in other rheumatologic conditions, including SLE and Kawasaki disease. Like other forms of HLH, MAS is characterized by fever, hepatosplenomegaly, cytopenias, and coagulopathy with the expansion of macrophages and T cells, as well as decreased cytotoxic T-cell and NK function. Approximately 10% of individuals with SJIA can develop life-threatening MAS, although it is believed that a much higher percentage may have a milder or subclinical form. Although MAS resembles HLH, diagnostic criteria for HLH may not apply because some features, such as hyperferritinemia, lymphadenopathy, and splenomegaly, often are present during a flare of the underlying disease. MAS generally responds to high-dose corticosteroids alone or in combination with cyclosporine.

Table 16-6 2004 revised diagnostic criteria for hemophagocytic lymphohistiocytosis

The diagnosis of HLH can be established if 1 of either item 1 or 2 is fulfilled:
1. A molecular diagnosis consistent with HLH
2. Diagnostic criteria for HLH fulfilled (5 out of the following 8 criteria)
Fever
Splenomegaly
Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood):
Hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L)
Platelets <100 × 10 ⁹ /L
Neutrophils <1.0 × 10 ⁹ /L
Hypertriglyceridemia and/or hypofibrinogenemia:
Fasting triglycerides ≥3.0 mmol/L (ie, ≥265 mg/dL)
Fibrinogen ≤1.5 g/L
Hemophagocytosis in BM, liver, lymph nodes or spleen
Low or absent natural killer cell activity (according to local laboratory reference)
Ferritin ≥500 µg/L
Soluble CD25 (ie, soluble IL-2 receptor) ≥2,400 U/mL

Langerhans-related histiocytoses (LCH)

Langerhans cells are specialized dendritic cells that are found in the skin and mucosa. Langerhans-related histiocytosis (LCH) is a neoplastic disorder of dendritic cells associated with polymorphic cellular infiltration and damage at either unifocal tissue sites or in multiple organs and tissues. Although the dendritic cells in LCH expresses similar antigens to skin Langerhans cells, including CD1a and CD207, they are believed to originate from a distinct myeloid dendritic cell precursor. Mutually exclusive somatic mutations in MAPK pathway genes, most commonly the *BRAFV600E* mutation, have been identified in >70% of patients LCH, revealing activation of extracellular

signal-regulated kinase (ERK) signaling as major driver of LCH pathogenesis.

LCH is rare, with an annual incidence of approximately 5 per million in children and 1 to 2 per million in adults. Patients with LCH are categorized as having either uni- or multifocal involvement of a single organ system (SS-LCH) or multisystem (MS-LCH). SS-LCH most commonly involves the bone (particularly the skull, femur, pelvis, and ribs) and less commonly the skin, lymph nodes, and lung. LCH of the lungs primarily occurs in adults and frequently is associated with smoking. Usual presentations of limited disease relate to the site of involvement and include persistent or recurrent and progressive bony pain or swelling, chronic skin rash, chronic ear drainage, dyspnea, cough, and pneumothorax. Diabetes insipidus may result from intracranial extension of craniofacial bone lesions and is the most common CNS manifestation, occurring in up to 30% of patients. MS-LCH most commonly occurs in young children and may present with various combinations of bony or soft tissue masses with symptoms including fever, eczematoid rash, gingival swelling, cough or dyspnea, tooth loss, hepatosplenomegaly, lymphadenopathy, abnormal chest x-ray, and cytopenias.

Tissue biopsy is required to confirm the diagnosis of LCH. Histologically, the lesions contain a mix of characteristic Langerhans cells in a background of eosinophils, neutrophils, and lymphocytes (Figure 16-7). Langerhans cells are positive for CD1a, S-100, and langerin (CD207), which confirms the presence of Birbeck granules specific to Langerhans cells. Some tumors contain an abundance of eosinophils and neutrophils with central necrosis, whereas fibrosis and foamy macrophages are found in more long-standing lesions.

Treatment of LCH is based on the extent and activity of the disease. SS-LCH generally confers a good prognosis and frequently requires minimal or no treatment. Bony or soft tissue SS-LCH can be treated with surgical resection or bony curettage, local irradiation, or injection of

steroids, unless multifocal (see below). Limited skin disease often responds to topical steroids, nitrogen mustard, or psoralen and ultraviolet A light therapy. Management of lung disease includes discontinuation of smoking; treatment with prednisone, vinblastine, and methotrexate; and immunosuppressive agents. Disease-free survival with limited or local LCH exceeds 95%; however, recurrences are common, and some patients require multiple courses of treatment to be cured. Therefore, patients must be monitored closely for evolution to multisystem disease, secondary malignancies, and, in the case of lung involvement, progressive pulmonary compromise.

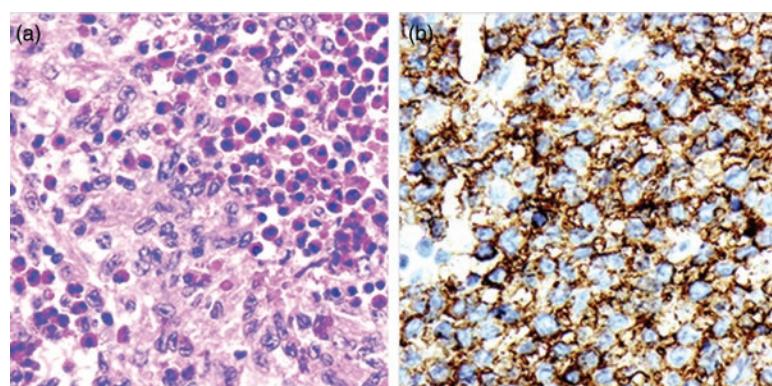
MS-LCH and SS-LCH with multifocal involvement or involvement of critical anatomic sites are treated with systemic therapy. Induction therapy with vinblastine and prednisone commonly is used as initial therapy. Involvement of the hematopoietic system, spleen, liver, and lung is considered high risk, with a mortality of ~20% compared with <5% for patients without high-risk features. Disease recurrence and progression are most common in patients with extensive visceral disease and a suboptimal initial response. In addition, long-term neurologic complications increasingly are being recognized in patients with LCH, particularly those with MS-LCH or CNS involvement. Neurodegenerative changes may be seen on MRI and can be accompanied by symptoms, including ataxia, dysarthria, dysmetria, and learning and behavior difficulties.

Erdheim-Chester disease (ECD) is the second major class of Langerhans-related histiocytoses. Historically, ECD was classified as a non-Langerhans cell histiocytosis; however, recent advances have revealed that, like LCH, the majority of ECD cases bear activating mutations in the MAPK pathway, most often *BRAFV600E*, leading to its reclassification as a myeloid neoplasm. In addition, ~20% of ECD patients have LCH lesions.

ECD is predominantly a disease of adulthood (mean age 55 years), although rare pediatric cases have been reported. There is a 3-to-1 male predominance. It is charac-

Figure 16-7 Langerhans cell histiocytosis.

(a) Hematoxylin-eosin stain demonstrating Langerhans cell infiltrate. Cells have abundant eosinophilic cytoplasm with variably shaped nuclei ranging from cleaved, grooved, folded, indented, and even lobated. Clusters of eosinophils surround the infiltrate. (b) CD1a immunohistochemistry staining Langerhans cells. From ASH Image Bank, images 3461 (a) and 3465 (b).



terized by tissue infiltration by foamy or lipid-laden histiocytes with associated fibrosis. ECD histiocytes are positive for CD68 and CD163, and, in contrast to LCH, are negative for CD1a and langerin, and only rarely positive for S100. Sites of involvement may include the skeleton, retroperitoneum, skin, CNS, heart, lungs, and less commonly lymph nodes, liver and spleen. More than 80% of patients have bilateral and symmetric diaphyseal and metaphyseal osteosclerosis of the legs, which is best visualized by bone scan or positron emission tomography (PET). In addition, ~60% and 40% of patients have dense infiltration of perinephric fat (so-called hairy kidney) and circumferential sheathing of the aorta (so-called coated aorta), respectively, on computerized tomography (CT). Additional clinical features may include xanthelasma, coronary infiltration, pericarditis, pericardial effusion, pseudotumoral infiltration of the right atrium, parenchymal CNS lesions, exophthalmos, and diabetes insipidus. Given the potential sites of involvement, recommended initial baseline assessments include CT chest, abdomen, and pelvis, PET/CT including distal extremities, MRI of the brain with contrast and detailed examination of the sella turcica, and cardiac MRI. Importantly, even in the presence of highly suggestive radiographic findings, biopsy is required to confirm the diagnosis and determine the *BRAF* mutational status. Given the therapeutic options for *BRAFV600E* mutated disease (see below) and the occurrence of false negative results with less sensitive methods, wild-type *BRAF* testing results should be confirmed by an additional genotyping method and/or genotyping of >1 anatomic site.

Given the rarity of ECD, there have been few prospective and no randomized clinical trials reported to date. Interferon- α has been associated with improved survival and is considered first line therapy for most patients. In addition, the *BRAFV600E* inhibitor, vemurafenib, is approved for ECD patients with *BRAF V600E* mutated disease. Response rates are high (~90%), and sustained on therapy, while treatment withdrawal is associated with relapse in the majority of cases. Because of the risks of adverse side effects, vemurafenib is currently recommended for those patients with *BRAFV600E* mutation who have moderate to severe disease or who have mild disease refractory to interferon- α or other conventional therapy.

Juvenile xanthogranuloma (JXG)

Juvenile xanthogranuloma (JXG), the most common of the cutaneous nonhistiocytoses, is a proliferative disorder presenting primarily in young children with solitary or multiple red, yellow, or brown papular skin lesions. The condition generally follows a benign clinical course and usually resolves spontaneously, although disseminated, ag-

gressive disease can rarely occur. Concurrent JXG and neurofibromatosis type I and JMML have been reported. Xanthogranulomas are rare in adults, but have been described in over 100 case reports, mostly involving the eye.

Rosai-Dorfman disease

Sinus histiocytosis with massive lymphadenopathy, also known as Rosai-Dorfman disease, is a nonmalignant proliferation of histiocytes within lymph node sinuses and lymphatics in extranodal sites. Emperipoleisis of intact lymphocytes and plasma cells by histiocytes is a hallmark of Rosai-Dorfman disease. The condition most commonly occurs in children and young adults and presents as massive, painless, bilateral lymph node enlargement in the neck with fever. Other nodal and extranodal sites may sometimes be involved. Although spontaneous resolution is observed in most cases, extranodal involvement often requires treatment, relapses can occur, and the condition occasionally can be fatal. There is no standard treatment approach, and therapies employed have included surgery, corticosteroids, radiation, thalidomide, or cytotoxic agents including vinca alkaloids and purine nucleoside analogues.

Emerging data indicate the presence of activating mutations in the MAPK/ERK pathway in a subset of JXG and Rosai-Dorfman disease cases, linking these non-Langerhans cell histiocytes with the Langerhans-related histiocytes through common dysregulated signaling.

KEY POINTS

- HLH is a pathologic activation and proliferation of tissue histiocytes leading to severe multisystem clinical consequences. HLH may present in young children with an inherited predisposition (eg, due to perforin gene mutations) or in children and adults with acquired disorders of immune regulation due to infection, autoimmune disorder, malignancy, or acquired immunodeficiency state.
- LCH is a clonal dendritic cell disorder that can present with involvement of a single tissue (usually the bone) or multiple tissues and organs, including the pituitary and hypothalamus (with diabetes insipidus). The clinical course may be variable, with periods of disease inactivity or chronic progression, and treatment must be individualized.

Lysosomal storage diseases

Lysosomal storage diseases are a collection of approximately 50 genetically inherited disorders characterized by a deficiency or defect in 1 or more specific lysosomal enzymes. These disorders lead to an accumulation of undigested

material inside the lysosome, leading to cell degeneration and accumulation of macromolecules in various tissues and organs of the body and resulting in organ dysfunction. Many present in infancy or early childhood with profound progressive neurologic abnormalities in addition to cytopenias; for example, Niemann-Pick disease (NPD). Gaucher disease represents a subtype of lysosomal storage diseases, also known as sphingolipidoses or lipid storage disorders, in which undigested lipids accumulate in the lysosome-rich cells of the monocyte or macrophage system. Gaucher disease is of particular importance to hematologists because type 1 patients most often present with cytopenias and hepatosplenomegaly, and are often managed by hematologists.

Gaucher disease

CLINICAL CASE

A 23-year-old man from Ukraine presents with a several-month history of easy bruising, worsening fatigue, and hip pain. On physical examination, the patient is noted to be pancytopenic, with marked hepatosplenomegaly. A BM biopsy reveals the presence of lipid-laden macrophages consistent with Gaucher cells infiltrating the marrow. Leukocyte glucocerebrosidase is reduced to <10% of normal levels.

Clinical, epidemiologic and genetic features

Gaucher disease is the most common lysosomal storage disease, resulting from deficiency of the glucocerebrosidase enzyme, which normally hydrolyzes glucocerebroside resulting from processing of senescent cells. This metabolite accumulates in the cytoplasm of macrophages in the BM, liver, spleen and other tissues, resulting in a diagnostic wrinkled-paper appearance of these Gaucher cells (Figure 16-8).

Gaucher disease is autosomal recessive, with an incidence of approximately 1 in 75,000 births, and is much more common in Ashkenazi Jewish populations than in other populations. The disease is divided into 3 clinical subtypes based on pattern and severity of neurologic

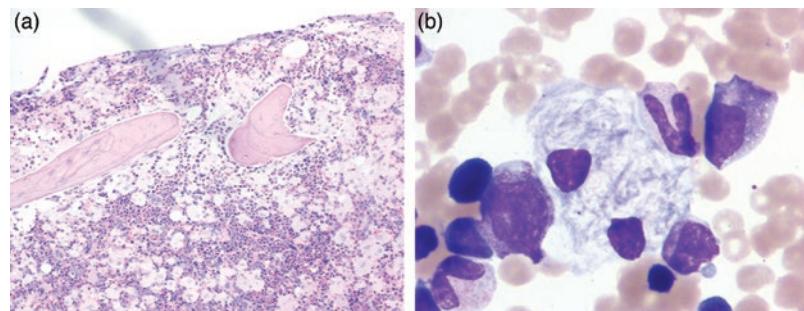
involvement. Type I (nonneuropathic) is most common (90% of all patients), has variable clinical presentation with onset of symptoms from 2 years of age to late adulthood, and is associated with the highest residual enzyme activity. Symptoms consist of hepatosplenomegaly, cytopenias, and bone deformation (flaring of the ends of the long bones and cortical thinning), and pain. Type II (acute neuropathic) is associated with the lowest enzyme activity, and results in progressive fatal neurologic deterioration beginning in infancy. Type III (subacute neuronopathic) falls between types I and II in incidence, enzyme activity, and clinical severity.

The diagnosis of Gaucher disease can be established by enzyme assay for glucocerebrosidase activity in leukocytes, fibroblasts, or urine, and should be decreased to 0% to 30% of normal values. Four specific mutations in the glucocerebrosidase gene account for 90% to 95% of Gaucher disease in the Ashkenazi Jewish population and 50% to 75% of the mutations in the general population, although over 300 mutations have been identified in patients with Gaucher disease to date. Patients with Gaucher disease have an increased risk of monoclonal gammopathies and multiple myeloma, and some have paraproteins reactive with the elevated glycosylceramides characterizing Gaucher disease. Both patients with Gaucher disease and carriers have a markedly elevated risk of Parkinson disease due to unclear pathophysiologic pathways linking lysosomal processing, mitochondrial function, and aggregate formation in the brain.

Treatment

Enzyme replacement therapy (ERT) is the mainstay of treatment for the nonneurologic manifestations of Gaucher disease. Imiglucerase is a recombinant glucocerebrosidase modified with mannose sugars to improve uptake and trafficking to the lysozymes of macrophages. ERT administered every 2 weeks normalizes cytopenias and reduces organomegaly within 6 to 12 months, although skeletal symptoms improve more slowly. Because glucocerebrosidase does not cross the blood-brain barrier, ERT has limited utility in the neuropathic forms of the disease.

Figure 16-8 Gaucher disease. (a) Proliferation of benign-appearing macrophages with interspersed normal hematopoietic elements. (b) High-power view of BM aspirate demonstrating a Gaucher cell, an abnormal macrophage with the characteristic “wrinkled-paper” cytoplasm. From ASH Image Bank, images 2711 (a) and 2709 (b).



A completely different treatment approach, termed oral substrate reduction (OSR) therapy, has been developed as an alternative to long-term ERT. Miglustat and eliglustat both inhibit glucosylceramide synthase, a key enzyme upstream of glucocerebrosidase, thereby reducing the substrate for the missing or dysfunctional glucocerebrosidase enzyme and decreasing toxic glucocerebroside accumulation. In clinical studies, improved platelet counts, decreased spleen and/or liver volume, and modest improvements in hemoglobin levels were achieved with OSR. OSR is usually reserved for patients unable to tolerate ERT, or those with mild disease.

Niemann-Pick disease

NPD is an autosomal recessive lysosomal storage disorder caused by mutations in the sphingomyelin phosphodiesterase-1 (*SMPD1*) gene, resulting in deficient sphingomyelinase activity and accumulation of sphingomyelin. Cytopenias and hepatosplenomegaly are common presenting manifestations to hematologists. Patients with type A present in early childhood and die of profound neurologic abnormalities within several years. Milder type B patients may present later in childhood or adulthood and, importantly, lack the neurologic feature observed in patients with type A NPD. The histologic hallmark of NPD is tissue accumulation of histiocytes filled with lipid droplets of uniform size, giving these foam cells a “mulberry-like” or “honeycomb-like” appearance. Currently, no specific treatment exists for NPD.

KEY POINTS

- Gaucher disease is a lysosomal storage disorder caused by mutations in the glucocerebrosidase enzyme, leading to abnormal accumulation of glucocerebroside in tissue macrophages and resulting in hepatosplenomegaly, cytopenias, and skeletal disorders.
- ERT and substrate reduction therapy can reverse both non-hematologic and hematologic manifestations of Gaucher disease

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Chronic myeloid leukemia

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The online version of this chapter contains an educational multimedia component on practical considerations for monitoring the response to TKIs in CML.

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Off-label drug use: Not applicable.

Overview, incidence, and prevalence

Chronic myeloid leukemia (CML) is a pluripotent hematopoietic stem cell neoplasm characterized by the *BCR-ABL* fusion gene, which is usually derived from a balanced translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11), resulting in a derivative chromosome known as the Philadelphia (Ph) chromosome. CML accounts for 15% to 20% of adult leukemia cases. The worldwide annual incidence of CML is one to two cases per 100,000 persons, with a slight male predominance (male-to-female ratio, 1.3:1). Because successful targeted therapy has returned life expectancy to that of the unaffected general population in many, the prevalence of CML continues to increase and is projected to reach 150,000 cases in the United States by 2040. In Europe, the median age of diagnosis ranges between 60 and 65 years, and in the United States, CML is most frequently diagnosed in individuals between the ages of 65 and 74. However, in countries where life span is shorter, the median age of diagnosis is substantially lower. CML in children and young adults is rare, constituting only 2% of all leukemias in children <15 years of age and 9% of all leukemias in adolescents 15 to 19 years of age. Radiation exposure has been implicated as a risk factor; however, unlike other myeloid leukemias, there has been no evidence for a causal association between CML and exposure to organic solvents, industrial chemicals, or alkylating agents.

Pathobiology

The Ph chromosome [der(22q)] was identified initially in patients with CML at what is now the Fox Chase Cancer Center in Philadelphia in 1960. As shown in Figure 17-1, the t(9;22)(q34;q11) translocation in CML juxtaposes the 3' segment of the *c-ABL* oncogene (normally encoding the Abelson tyrosine kinase [TK]) from the long arm of chromosome 9 to the 5' part of the breakpoint cluster region (*BCR*) gene on the long arm of chromosome 22. The resultant hybrid oncogene is transcribed as a chimeric *BCR-ABL1* mRNA, which, in turn, is translated into a functional abnormal protein. At diagnosis, the characteristic t(9;22)(q34;q11) is present in approximately 95% of CML cases. The remaining cases have either variant translocations involving a third and sometimes a fourth chromosome or cryptic translocations. In these cases, routine cytogenetic

CLINICAL CASE

A 60-year-old male construction worker with a history of coronary artery disease and hyperlipidemia came to see a physician for persistent fatigue of 2 months' duration. He complained of intermittent episodes of palpitations, dizziness, weight loss, and discomfort in the left upper quadrant of the abdomen. Physical examination was remarkable for palpable splenomegaly measuring 4 cm below the left subcostal margin. Routine complete blood count showed leukocytosis (white blood cells [WBCs] = $40 \times 10^9/L$) with predominance of neutrophils and neutrophil precursors (8% myelocytes, 4% metamyelocytes), normocytic anemia (hemoglobin = 10.2 g/dL, hematocrit = 35%, mean corpuscular hemoglobin = 85 fL), and an elevated platelet count (platelets = $435 \times 10^9/L$). Also noted on laboratory examination were basophilia (4%), eosinophilia (3%), and blasts (1%). A bone marrow aspirate and biopsy were performed and showed a hypercellular marrow (100% cellularity) with granulocytic proliferation. Metaphase cytogenetics showed t(9;22) (q34;q11) [20] in all cells, but no other additional cytogenetic aberrations were detected. Reverse transcriptase-quantitative polymerase chain reaction (RT-QPCR) for BCR-ABL1 mRNA transcripts on the International Scale (IS) in the peripheral blood was 69%. The Sokal risk score was calculated at 0.94 (intermediate risk).

analysis may be unable to detect the Ph chromosome, and the diagnosis relies on demonstration of either gene fusion by interphase fluorescence in situ hybridization (FISH) or the fusion transcript by reverse transcriptase-polymerase chain reaction (RT-PCR).

Three separate breakpoint regions in the *BCR* gene are associated with distinct disease phenotypes. In typical CML, the *BCR* gene is interrupted between exon 13 (e13) and e14 or between e14 and e15. Collectively, the region containing exons 12 to 16 is referred to as the major breakpoint cluster region (M-BCR). In a rearrangement involving M-BCR, the 5' *BCR* segments on chromosome 22 are joined with the sequences from *c-ABL* that are 3' from the a2 breakpoint (a breakpoint near the 5' end of *c-ABL*). This union gives rise to hybrid transcripts called e13a2 (also known as b2a2) or e14a2 (b3a2). These transcripts are translated into 210-kDa proteins, collectively known as p210 BCR-ABL1. Importantly, the rearranged *c-ABL* segment here includes sequences necessary for TK activity. As a result, the p210 BCR-ABL1 oncoprotein functions as a constitutively active TK that can phosphorylate a number of cytoplasmic substrates with other activities of the chimeric protein, leading to alterations in cell proliferation, differentiation, adhesion, and

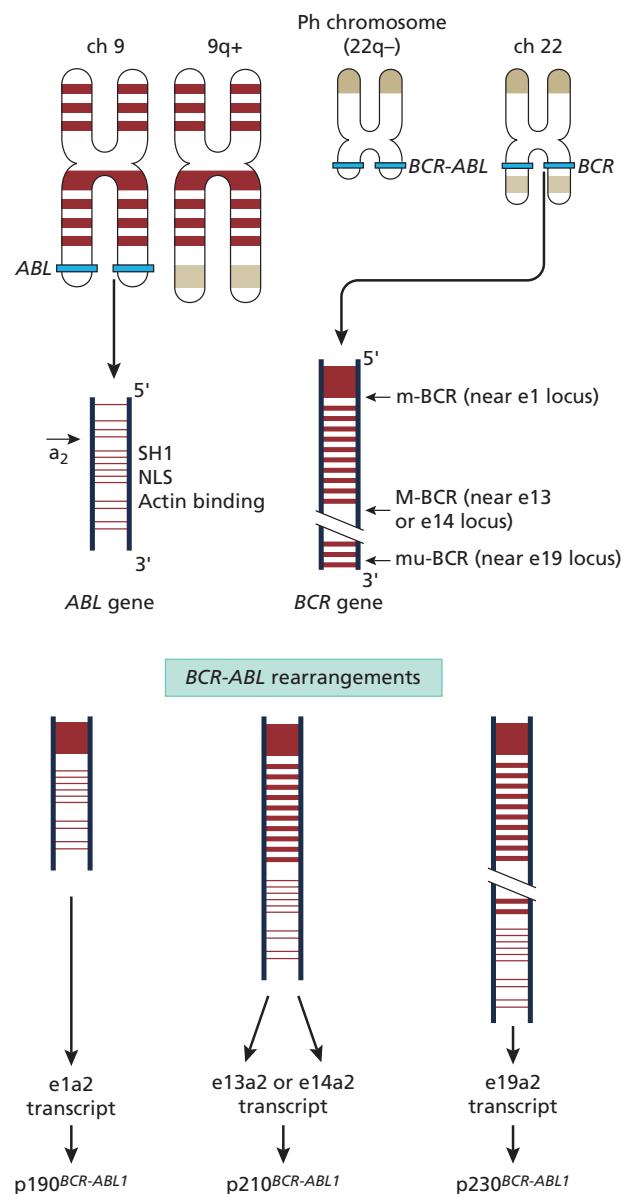


Figure 17-1 Molecular pathogenesis of t(9;22)(q34;q11) in CML. The 3' portion of the *ABL* gene on the telomeric region of the long arm of chromosome 9 is translocated to the *BCR* gene on chromosome 22 to form the characteristic 22q- abnormality referred to as the Philadelphia (Ph) chromosome. Breakpoints in the *ABL* gene occur in intron 1b or 2, both of which are 5' (upstream) to the a2 exon. The a2 and downstream exons of *ABL* encode the Src homology (SH) domains of the *ABL* kinase, including the SH1/tyrosine kinase domain, DNA binding domain, nuclear localization signal (NLS), and actin binding site. The breakpoints on chromosome 22 occur at one of three locations in *BCR*, yielding hybrid oncogenes of varying length consisting of 5' *BCR* sequences and 3' *ABL* sequences. Each hybrid oncogene gives rise to a chimeric transcript, which encodes a fusion protein with oncogenic activity. These include p190^{BCR-ABL1} (resulting from fusion at the minor breakpoint or m-BCR site), the p210^{BCR-ABL1} gene product (resulting from fusion at the major breakpoint or M-BCR site), and p230^{BCR-ABL1} (resulting from fusion at the micro breakpoint or mu-BCR site).

survival. Studies have suggested that patients with e13a2 transcripts, as compared to e14a2 transcripts, take longer to achieve major molecular response (MMR) and that transcript type may influence progression-free survival (PFS) and failure-free survival.

Two alternative types of translocation involving *BCR* and *ABL* also have been implicated in the pathogenesis of hematologic malignancies (Figure 17-1). In one of these, a similar segment of *c-ABL* is transposed onto a locus of *BCR* that is downstream from the M-BCR locus, a region referred to as mu-BCR (exons 17 to 20). Translocations involving mu-BCR yield a larger fusion gene than those involving M-BCR, and one such fusion (e19a2) gives rise to a 230-kDa p230 BCR-ABL1 protein. The p230 BCR-ABL1 product has been found uncommonly in CML variant cases that are characterized by chronic neutrophilia with or without thrombocytosis. These cases tend to have a more indolent disease course than CML associated with p210 BCR-ABL1.

The third type of *BCR-ABL1* rearrangement juxtaposes the same *c-ABL* segment to the minor *BCR* break-point region (m-BCR), which is located upstream from the M-BCR (exons 1 and 2). The resultant smaller chimeric oncogene generated by this rearrangement gives rise to a 190-kDa p190 BCR-ABL1 protein product. The p190 BCR-ABL1 transforming protein is most often found in *de novo* acute lymphoblastic leukemia (ALL) cases referred to as Ph-positive ALL. Sometimes, the p190 BCR-ABL1 product can be detected in CML, either coexpressed with p210 BCR-ABL1 (5% to 10% of cases) or detected alone in atypical cases that are often associated with monocytosis. Coexpression of p190BCR-ABL1 and p210BCR-ABL1 is attributed to alternative splicing of the transcript arising from the M-BCR chimeric oncogene.

The leukemic clone in CML has a tendency to acquire additional oncogenic mutations over time. Clinically, the acquisition of additional cytogenetic aberrations (ACAs) or molecular abnormalities is associated with progression to accelerated and blast phases of disease or resistance to TK inhibitors (TKIs). In some cases, these ACA are also present at diagnosis and have a variable influence on prognosis. A study of 1,151 patients in the German CML study IV identified that major-route ACAs [trisomy 8, amplification of t(9;22), isochromosome 17q, or trisomy 19] were associated with poorer PFS and overall survival (OS) as compared to patients without ACA or with rarer ACAs (called minor-route ACAs). Other studies have suggested that trisomy 8 may not be associated with poorer response, while isochromosome 17q, -7/del7q, and 3q26.2 are associated with poorer treatment responses and OS.

At the molecular level, mutations in the kinase domain of *BCR-ABL1* can emerge. Resistance to TKI therapy is

often characterized as primary or secondary (ie, acquired) resistance. The etiology of primary resistance remains largely unknown, but reported mechanisms include altered drug transport and BCR-ABL-independent mechanisms (where BCR-ABL remains inhibited, but disease is not significantly altered or disease progression occurs). Although point mutations in the ABL tyrosine kinase domain (TKD) are rarer in primary resistance, they are a common cause of acquired TKI resistance and the incidence of mutations increases in advanced disease. Approximately 25% of patients in chronic phase (CP) who develop resistance to imatinib have an ABL TKD mutation. Importantly, identification of a TKD mutation can influence treatment selection after an inadequate response and/or TKI resistance is encountered. Mutations are currently detected using technology involving Sanger sequencing, where the clone affected by the mutation forms at least 20% of the residual leukemia. More than 80 point mutations have been described after imatinib exposure, but substitutions at seven amino acid residues (G250, Y253, E255, T315, M351, F359, and H396) comprise ~60% of mutations reported in larger surveys. Subsequent TKI generations have been designed to minimize resistance due to mutations. Dasatinib resistance-associated mutations include T315I, F317L/V/I/C, and V299L. The Y253H, E255K/V, T315I, and F359V/C/I mutations are associated with nilotinib resistance, and L248V, G250E, V299L, T315I, and F359C are associated with bosutinib resistance. Ponatinib treats CML with any mutation, including T315I; rare compound mutations (ie, mutations on the same DNA strand) have been described but may not contribute significantly to ponatinib resistance. The importance of lower-level mutations identified by newer methodologies of next-generation sequencing is currently under investigation by a number of groups.

Diagnosis

The majority of CML patients present with CP disease, most commonly with an insidious onset, and are diagnosed based on abnormalities observed on complete blood count. Common symptoms at presentation can include fatigue, night sweats, weight loss, and gout attacks. Many patients also present with splenomegaly (50% to 90%) at diagnosis, which may be symptomatic. Thrombotic and hemorrhagic complications are relatively infrequent (<5%), although purpura is a common complaint. Hyperleukocytosis alone does not routinely cause symptoms because of the relative maturity of the leukemic cells and their smaller size compared with the immature, large, poorly deformable blast cells seen in acute leukemia; however, in rare cases, patients can present with visual disturbances, including retinal hemorrhage, and males with very high WBC counts can

present with leukostasis-related priapism. A progressively severe symptom burden, marked by constitutional symptoms, including fever, night sweats, weight loss, bleeding, bone pain, and worsening splenomegaly, may herald onset of accelerated-phase (AP) or blast-phase (BP) CML, defined below.

In the peripheral blood, neutrophilia and immature circulating myeloid cells are hallmark features of CP CML. More than 50% of patients present with a WBC count of $>100 \times 10^9/L$, with blasts usually accounting for <2% of the WBCs. Absolute basophilia is usually present, and eosinophilia is common. Anemia may be present in up to one-half of patients. Roughly 15% to 35% of patients present with platelet counts of $>700 \times 10^9/L$, although extreme thrombocytosis (ie, $>1,500 \times 10^9/L$) is uncommon. The high cell turnover and hypercatabolic state of CML

are associated with elevated lactate dehydrogenase and uric acid levels.

The marrow in CP CML typically shows myeloid hyperplasia and an elevated myeloid-to-erythroid ratio (often >10:1). Bone marrow blasts are <10%. Maturation of precursors is normal in CML, and dysplastic features are not routinely found. Megakaryocytes are often smaller than normal, in contrast to large megakaryocytes that can be seen in other myeloproliferative neoplasms, and show hypolobation, clustering, and peritrabecular localization. Marrow basophilia is noted in one-fourth of cases. A progressive symptom burden and change in laboratory characteristics mark progression to AP or BP CML; these abnormalities are summarized in Table 17-1.

A suspected case of CML can be confirmed by assays of the peripheral blood to detect either the *BCR-ABL1*

Table 17-1 Clinicopathological features of chronic-, accelerated-, and blast-phase CML

Symptom	Blood and bone marrow findings (WHO classification)
Chronic phase	
Fatigue	Neutrophilic leukocytosis with immaturity
Weight loss	Peripheral blasts <10%
Nocturnal sweats	Thrombocytosis
Left upper-quadrant abdominal pain	Basophilia and/or eosinophilia
Early satiety	Normocytic anemia
Palpitations and/or dyspnea	<i>BCR-ABL1</i> rearrangement (usually p210 BCR-ABL1, may be e13a2 or e14a2 variants or both)
Bleeding/bruising	High lactate dehydrogenase
Priapism	Hyperuricemia
	Marrow myeloid and megakaryocytic hyperplasia, mild/moderate fibrosis, <10% blasts, minimal dysplasia,
	t(9;22) ± other abnormalities
Accelerated phase (several definitions exist)	
Unexplained fever or bone pain, progressive weight loss, and sweats	Increasing WBC count unresponsive to therapy
Increasing spleen size (can also result in splenic infarction)	Peripheral blood basophils ≥20%
	Persistent thrombocytopenia ($<100 \times 10^9/L$) unrelated to therapy, or persistent thrombocytosis ($>1,000 \times 10^9/L$) unresponsive to therapy
	Blasts 10% to 19% of WBCs in peripheral blood and/or nucleated bone marrow cells
	Cytogenetic evidence of clonal evolution
Blast phase	
Bleeding, bruising, bone pain	Blasts ≥20% of peripheral blood white cells or of nucleated bone marrow cells
Infections	Extramedullary blast proliferation
Prominent constitutional symptoms	Large foci or clusters of blasts in the bone marrow biopsy
Massive splenomegaly	
Tissue manifestations of extramedullary disease	

fusion gene at the chromosome level or its chimeric transcripts. At diagnosis, the sensitivity of FISH or RT-PCR of peripheral blood is equal to that of bone marrow. FISH allows for identification and quantitation of the chimeric oncogene among interphase nuclei on a peripheral blood smear; usually, 200 to 500 nuclei are screened. RT-PCR is carried out on peripheral blood-derived RNA and is an extremely sensitive technique; RT-PCR can detect the *BCR-ABL1* transcript in fewer than 1 of 10^5 cells in most laboratories. Both methods can detect “masked” or cryptic chromosomal translocations that are missed by conventional cytogenetics in ~5% of cases. FISH has the advantage of identifying unusual variant rearrangements that are outside the regions amplified by the RT-PCR primers. The RT-PCR method, unlike FISH, can differentiate between the fusion genes encoding the p210 *BCR-ABL1* product and the p190 *BCR-ABL1* product. Additionally, RT-PCR provides more accurate detection and quantification when disease levels are low. Because of the lower cost, ability to discriminate breakpoints, and accurate quantitation at low levels of disease burden, RT-QPCR is becoming the preferred assay for CML diagnosis and monitoring.

Although a positive RT-PCR or FISH assay in the peripheral blood confirms the diagnosis of CML, a complete staging of the disease still requires a bone marrow evaluation (Table 17-1). A marrow sample at diagnosis will provide an assessment of the percentage of blasts and identify ACAs, which impact prognosis and allows for correct staging of the disease. Conventional cytogenetic studies identify a Ph chromosome in 90% to 95% of cases; more than one-half of the karyotypic negative cases have a detectable *BCR-ABL1* rearrangement by molecular assay. The clinical course of *BCR-ABL1*-positive, Ph chromosome-negative patients is identical to that of patients with Ph-positive CML. The presence of variant translocations or deletion of the derivative chromosome 9 (der 9q del), do not appear to impact cytogenetic or molecular response or outcomes on imatinib, and der 9q del does not appear to impact outcomes on nilotinib or dasatinib.

Disease course and prognosis

CP CML

Historically, patients diagnosed with CP CML remained stable for an average of 3 to 5 years before progressing to AP or BP CML. Before the development of TKIs, patients with CML who did not undergo stem cell transplantation (SCT) had a median survival of roughly 5 to 7 years, and 30% of patients survived beyond 10 years. Recent updates to the phase 3 International Randomized Study of Interferon and

STI571 (IRIS) study, which resulted in regulatory approval of imatinib in 2001, highlight that prognosis has changed dramatically in the era of TKIs. With a median follow-up of 10.9 years, the OS of patients treated with imatinib was 83.3%. When the analysis was limited to CML-related deaths, the estimated survival rate at 10 years was 97.8% in patients who had achieved an MMR (<0.1% *BCR-ABL1* IS). Survivals for patients in the IRIS study were similar to the OS reported for patients treated with imatinib-based regimens in the German CML Study IV (84%).

Before the development of TKIs, multivariate prognostic models (eg, the 1984 Sokal score, including age, spleen size, platelet counts, and percent blasts) and the 1998 Hasford (Euro) score (added eosinophil and basophil percentage to Sokal score) were useful to help identify patients at high risk of treatment failure. Colleagues from the European LeukemiaNet (ELN) have attempted to improve upon these scores using large cohorts of patients treated with TKI from diagnosis. The European Treatment and Outcome Study for CML (EUTOS) score was developed to predict complete cytogenetic response (CCyR) at 18 months and has not proved to be a consistent predictor of OS or PFS. This may reflect the fact that CML per se is now a rare cause of death and patients are more likely to succumb to other medical conditions. The EUTOS long-term survival score has recently been developed to try to predict death from disease. Although these scoring systems are useful, particularly in the context of choosing first-line therapy, the most important prognostic indicators remain phase of disease at diagnosis and the speed and depth of response to TKI therapy. Notably, prognostic risk scores have not been validated in children and may not apply. Appropriate monitoring strategies continue to evolve and are discussed subsequently.

AP CML

The AP CML is accompanied by the acquisition of additional molecular lesions, genomic instability, and progressive impairment of myeloid cell differentiation. This latter feature leads to the accumulation of immature precursors and blasts in the marrow, blood, and extramedullary tissue. The clinical symptoms associated with AP CML (Table 17-1) may be minor, delayed, or completely absent. The World Health Organization (WHO) definitions of AP are shown in Table 17-1. It should be noted, however, that the MD Anderson and ELN definitions have been used to define CP and AP CML for most clinical studies reported in this chapter. Although, the definitions are generally similar, the proportions of blasts in AP are 15% to 29% and 10% to 19% in the ELN/MD Anderson and WHO criteria, respectively. For the majority of clinical

trials, ELN/MD Anderson criteria were used to define phase. In the absence of effective therapy with either TKI or allogeneic SCT, the median survival from the onset of AP, historically, is only 12 to 18 months. Death occurs predominantly because of transformation to BP with the associated life-threatening complications of marked leukocytosis and complete failure of normal hematopoiesis. It has also been observed that the proportion of pediatric patients diagnosed with AP or BP is higher than that for adult patients, although the reasons for this observation are unclear. Although AP patients do not share the generally good prognosis of CP patients in the era of TKIs, studies of newly diagnosed AP patients, as defined by ELN criteria, have identified subsets of patients who may respond well to first-line TKI therapy, which is discussed in a later section.

BP CML

Progression of CML to acute leukemia, synonymous with “blast phase” or “blast crisis,” evolves most commonly from a preceding AP and is reached when the proportion of blasts in the blood or marrow is >20% (Table 17-1) (WHO criteria). It should be noted, however, that the majority of clinical trials used ELN/MD Anderson criteria to define BP ($\geq 30\%$ blasts). Data from the IRIS study demonstrated that the risk for progression to AP or BP is highest in the first 4 years of imatinib treatment and reported annual rates of progression in years 1 to 4 of 1.5%, 2.8%, 1.6%, and 0.9%, respectively. Myeloid lineage markers (eg, CD33, CD13, CD14, and CD15) are expressed by the blast cells in more than one-half of the cases of BP CML. Up to one-third express B-cell-precursor lymphoid markers (eg, CD10, CD19, and CD20). Undifferentiated acute leukemia cases displaying both myeloid and lymphoid cell surface markers account for the remainder. Most CML cases express the p210 *BCR-ABL1* gene product, and only rare cases are associated with p190 *BCR-ABL1* alone. Thus, a case of Ph-positive ALL that subsequently is found to be associated with p210 *BCR-ABL1* might actually represent previously unrecognized CML presenting in lymphoid BP. That said, the diagnosis of lymphoid BP typically relies on documentation of a preceding CP. The clinical and laboratory features of BP CML are summarized in Table 17-1. Although BCR-ABL is still an important driver, BP cells acquire additional cytogenetic and molecular changes contributing to either poor TKI response or rapid loss of response. ACAs in addition to t(9;22) are found in 65% to 80% of cases of BP. Unfortunately, even in the era of TKIs, outcomes for BP CML remain poor, with median survival ranging between 7 and 11 months. The presence of >50% blast cells in the

blood and cytogenetic progression have been identified as independent predictors of worse survival. Deaths usually are due to metabolic derangements, infection, bleeding, and extramedullary leukemic infiltration.

TKI response criteria

The development of TKIs has completely changed the standard therapeutic approach for all phases of CML, and response to these therapies has a substantial impact on prognosis. As such, response to therapy and many clinical trial endpoints are measured by meeting certain treatment responses or “milestones” at particular times in the treatment course. Criteria for complete hematological response (CHR) include resolution of symptoms and signs of the disease, including palpable splenomegaly, leukocytes $<10 \times 10^9/L$ and absence of immaturity (myelocytes, promyelocytes, blasts, etc.), and platelets $<450 \times 10^9/L$. CCyR is achieved if there are no Ph-positive metaphases, whereas partial cytogenetic response is characterized by 1% to 35% Ph-positive metaphases, major cytogenetic response (MCyR) by 0% to 35% Ph-positive metaphases (complete plus partial), and minor responses by >35% Ph-positive metaphases. Molecular responses are reported as a percentage of the ratio of *BCR-ABL1* transcripts to those of a control gene. Two common control genes are *ABL1* and *BCR*. Peripheral blood is the preferred source, not only due to ease of sampling, but also because it has been shown to correlate with results from bone marrow samples and because the majority of clinical trial data have been reported from peripheral blood measurements. IS was developed to harmonize molecular responses across laboratories. IS response is derived by applying a laboratory-specific conversion factor to molecular response data from each individual participating laboratory. This conversion factor is derived from comparison to a reference laboratory and is monitored over time for “drift” in IS measurements. All molecular response criteria and recommendations for intervention in the National Comprehensive Cancer Network (NCCN) or ELN guidelines are based upon IS molecular response. It is important to note that the ability to report specific depths of response is dependent on the quality of the control mRNA values. An MMR is defined as *BCR-ABL1* IS transcripts of 0.1% or less. Deep molecular responses (MRs) include MR 4.0 (*BCR-ABL1* $\leq 0.01\%$) and MR 4.5 (*BCR-ABL1* $\leq 0.0032\%$). Definitions of response are shown in Table 17-2. Early molecular response (EMR; *BCR-ABL1* transcripts $\leq 10\%$) at 3 months is associated with good prognosis, and treatment guidelines recommend that *BCR-ABL1* transcripts $>10\%$ be considered a warning and are a trigger to examine patient adherence and assess for resistance.

Table 17-2 Definitions of response

Response	Definition
CHR (complete hematologic response)	Leukocyte count $<10 \times 10^9/L$; platelet count $<450 \times 10^9/L$; normal differential with no early forms; no splenomegaly
MCyR (major cytogenetic response)	0% to 35% Ph-positive metaphases (marrow)
PCyR (partial cytogenetic response)	1% to 35% Ph-positive metaphases (marrow)
CCyR (complete cytogenetic response)	0% Ph-positive metaphases (marrow)
MMR (major molecular response)	<i>BCR-ABL1</i> IS $\leq 0.1\%$
MR (deep molecular response)	<i>BCR-ABL1</i> IS ≤ 0.01 (MR 4.0)
	<i>BCR-ABL1</i> IS ≤ 0.0032 (MR 4.5)
	Undetectable <i>BCR-ABL1</i> (assay sensitivity ≥ 4.5 logs)

Abbreviations: Ph is Philadelphia chromosome; *BCR-ABL1* IS refers to percentage *BCR-ABL1*/control gene, standardized to the International Scale. Common control genes are *ABL1* and *BCR*.

TKI therapy, management, and monitoring in chronic phase

Imatinib mesylate

The promise of targeted therapy for CML was realized with the regulatory approval of the first small-molecule TKI for cancer, imatinib mesylate, in May 2001. Imatinib binds the adenosine triphosphate binding site in the catalytic domain of the BCR-ABL1 oncprotein and inhibits the BCR-ABL1 TK activity. This interaction prevents the transfer of phosphate groups to tyrosine residues on substrate molecules involved in downstream signal transduction pathways. The drug also interferes with the TK activities of normal ABL and with the kinase activity of the ARG, PDGFRA, PDGFRB, and KIT TKs. These actions are useful for the treatment of other hematopoietic disorders (eg, systemic mastocytosis without KIT mutations, chronic eosinophilic leukemia), dermatofibrosarcoma protuberans and other tumors (eg, gastrointestinal stromal tumor). Generic imatinib is now available.

The pivotal phase 3 study comparing imatinib to the combination of interferon alpha (IFN α) and cytarabine (IRIS study) demonstrated the superiority of imatinib compared with IFN α plus cytarabine, with higher rates of CHR, MCyR, and CCyR; freedom from progression to AP or BP CML; and better tolerance of therapy. With a median follow-up of 19 months, this study reported estimated rates of CCyR of 76.2% for imatinib-treated patients vs 14.5% for patients receiving IFN α and cytarabine. A recent 10-year follow-up report provided long-term efficacy and safety data on 553 patients who were randomized to the first-line imatinib arm of the IRIS study. At the end of the trial, the rate of CCyR at any time was 82.8%. Among patients with evaluable molecular data at 10 years (N=204/516), 93.1% had

achieved MMR and 63.2% MR 4.5. The estimated OS at 10 years was 91.1% vs 85.3% in patients with and without MMR, respectively, at 12 months. There were low yearly rates of progression to AP or BP CML in years 4 to 8 after starting imatinib treatment (0.9%, 0.5%, 0%, 0%, and 0.4%). Among the imatinib-treated group, 6.9% had progression to AP or BP and the estimated rate of freedom from progression to AP or BP at 10 years was 92.1%. Estimated OS at 10 years was worse in patients with a high Sokal risk score as compared to those with an intermediate or low risk score (68.8% vs 80.3% vs 89.9%, respectively). Among patients randomly assigned to imatinib, 15.9% of patients discontinued study treatment due to unsatisfactory therapeutic effect and 6.9% due to adverse events.

Despite impressive results with imatinib, several attempts have been made to improve response rates and decrease resistance in newly diagnosed patients through the use of higher doses of imatinib (600 to 800 mg/d). The rationale for use of higher-dose imatinib is based on interpatient variability of drug uptake into target hematopoietic cells, which itself depends, in part, on human organic cation transport-1 activity, and early clinical data demonstrating higher rates of CCyR and of molecular response in patients with newly diagnosed CP CML. Phase 2 studies demonstrated that higher-dose imatinib yields higher rates of CCyR and MMR at earlier time points for newly diagnosed low—or intermediate—Sokal risk CML patients.

A number of groups have examined the effect of either increasing the starting dose of imatinib or adding IFN α or cytosine arabinoside (Ara-C) to standard dose imatinib. The phase 3 Tyrosine Kinase Inhibitor Optimization and Selectivity (TOPS) study compared high-dose (800 mg/d) with standard-dose imatinib (400 mg/d) and showed higher rates of CCyR and MMR at 6 months, but

not at subsequent months. Higher rates of adverse events in the high-dose arm resulted in approximately 50% of patients reducing their dose to 600 mg daily or less. Furthermore, this study, together with an Italian trial specifically focused on patients with high-risk Sokal scores, was unable to show any differences in the rates of CCyR and MMR at 12 months between the two treatment arms based on Sokal risk scores. Lastly, when comparing high-dose to standard-dose imatinib at 48 months, no differences in estimated event-free survival (EFS), PFS, and OS were found. Finally, the TIDEL-II study provided evidence to support that an imatinib-based initial therapy approach with an early switch may be practical and effective. This study evaluated higher-dose imatinib (600 mg daily), followed by dose escalation to 800 mg daily if the plasma trough at day 22 was below target levels. Thereafter, if patients failed to meet molecular targets ($BCR-ABL1 \leq 10\%$, $\leq 1\%$, and $\leq 0.1\%$ at 3, 6, and 12 months), they were either increased to imatinib 800 mg daily and later to nilotinib if failing the same target (cohort 1) or directly to nilotinib (cohort 2). At 2 years, 55% and 30% remained on imatinib and nilotinib, respectively, only 12% failed to achieve EMR at 3 months, and MMR was 73% (4.5-log reduction 34%).

Two large European studies randomized newly diagnosed patients to standard-dose imatinib with or without IFN or Ara-C or to higher-dose imatinib. The French SPIRIT study showed that adding pegylated IFN alfa-2b resulted in higher rates of MMR (82% vs. 54%, $P=.002$) compared to imatinib alone, but the rate of IFN discontinuation was 61% in the combination group. The German CML Study IV randomized 1,551 patients to imatinib 400 mg/d, 800 mg/d, 400 mg/d plus IFN, 400 mg/d plus Ara-C, or the use of imatinib after IFN failure. The 5-year OS and PFS rates were 90% and 87.5%, respectively, for the entire cohort. MR4.5 was reached more quickly with optimized high-dose imatinib than with imatinib 400 mg/d ($P=.016$) and was associated with a higher survival probability than the achievement of CCyR only (8-year OS, 92% vs 83%; $P=.047$). At 10 years, OS, PFS, and CML relative survivals were 82%, 80%, and 92%, respectively. Survival between imatinib 400 mg and any experimental arm was not different. In a multivariate analysis, disease risk group, major-route ACA, comorbidities, smoking, and treatment center (academic vs other) influenced survival significantly, but not any form of treatment optimization. Survival, irrespective of treatment arm, was significantly better for patients who achieved $BCR-ABL1 \leq 10\%$ at 3 months, $\leq 1\%$ at 6 months, or $\leq 0.1\%$ at 12 months. Currently, neither high-dose imatinib nor imatinib in combination with IFN are recommended frontline treatments and would be considered investigational.

Toxicity

Adverse effects include myelosuppression (in particular neutropenia), fatigue, gastrointestinal disturbances such as nausea and diarrhea, rash, edema (periorbital and peripheral), and muscle cramps. Long-term consequences may rarely include hypophosphatemia and a decrease in bone mineral density. Cardiotoxicity, including congestive heart failure, is rare. For children, unique toxicities exist, including growth abnormalities, especially in prepubertal children. These effects may be due to effects on the growth hormone/IGF-1 axis. The long-term safety profile of imatinib remains excellent. In many patients who experience unacceptable adverse effects, transient dose reduction or treatment interruption with supportive care allows patients to resolve adverse effects and resume full-dose or modified therapy. An excellent review of side effects on all TKIs and management of these side effects was recently published by ELN.

Dasatinib

Dasatinib, which lacks structural similarity to imatinib, has activity against Src family kinases in addition to ABL kinases. Dasatinib does not rely on a conformational change of ABL for binding and thus appears to be less susceptible to the development of resistant kinase domain mutations that alter ABL conformation. Dasatinib is approved for the treatment of adults with newly diagnosed CP CML and CP CML with resistance or intolerance to prior therapy.

Frontline therapy

Data from the 3- and 5-year follow-ups of patients enrolled in the phase 3 randomized, open-label trial Dasatinib versus Imatinib study in Treatment-Naïve CML-Chronic Phase (DASISION) showed that CCyR rates between dasatinib- and imatinib-treated patients were 87% vs 83%, but the median time to CCyR was shorter in dasatinib-treated patients (3.1 months vs 5.8 months). The cumulative rates of MMR and deeper responses including MR4.0 and MR4.5 were higher for dasatinib as compared to imatinib. Transformation to AP or BP occurred in 5% and 7% of patients in the dasatinib and imatinib arms, respectively. More imatinib-treated patients died because of CML-related causes ($N=17$) compared with dasatinib-treated patients ($N=9$); however, the related 5-year OS was not statistically significantly different at 91% for dasatinib and 90% for imatinib (HR, 1.01; 95%CI, 0.58 to 1.73). In patients who achieved EMR ($BCR-ABL1 \leq 10\%$) at 3 months (dasatinib, 84%; imatinib, 64%), improvements in PFS and OS and lower rates of transformation to AP/BP were reported compared with patients not achieving EMR at 3 months.

Second-line therapy (after imatinib resistance or intolerance)

Dasatinib was first investigated in CML patients with resistance or intolerance to imatinib in a series of phase 2 trials called START (SRC/ABL Tyrosine kinase inhibition Activity Research Trials). The START-C study was a single-arm study of dasatinib at 70 mg orally twice daily, and START-R was a randomized parallel-arm study of dasatinib vs high-dose imatinib. For START-C, MCyR and CCyR rates were 62% and 53%, respectively, with a minimum follow-up of 24 months. Results for START-R were similar. Additionally, START-R demonstrated superior MCyR and CCyR rates for the use of dasatinib rather than an increased dose of imatinib. Notably, for both studies, the median daily dose was ~100 mg daily due to dose reductions. Consequently, a phase 3 dose-optimization study randomized patients 1:1:1:1 between four dasatinib treatment groups: 100 mg once daily, 50 mg twice daily, 140 mg once daily, or 70 mg twice daily. Seven-year follow-up from this study for patients receiving dasatinib at 100 mg daily demonstrated sustained benefit, with MMR, PFS, and OS rates of 46%, 42%, and 65%, respectively. Similar to first-line studies, EMR was associated with improved PFS and OS. Across dasatinib studies for CP, as well as advanced phase, treatment responses were limited for patients with T315I or F317L mutations, and possibly lower response rates were seen in patients with Q252H, E255K, or E355G mutations.

Toxicity

Adverse effects of dasatinib include myelosuppression, in particular neutropenia and thrombocytopenia. Unique toxicities include pleural effusion, suggesting that patients with lung disease, congestive heart failure, and hypertension may not tolerate this agent. The incidence of pleural effusion increases with increasing dose and age. With 7-year follow-up of the dose optimization study, the incidence of pleural effusion was 28% at 100 mg once daily vs 35% for the other dose groups and was similar to the incidence reported in updates at 5 years from the first-line DASISION study. Other unique, but uncommon, toxicities include pulmonary hypertension and platelet dysfunction. The incidence of pulmonary hypertension is reported to be ≤5% and often occurs concurrently with pleural effusion. A recent retrospective review of 41 cases suggests pulmonary hypertension may be reversible, in part, with dasatinib cessation. Lastly, reports suggest dasatinib use has effects on growth in children similar to imatinib.

Nilotinib

Nilotinib is a structural derivative of imatinib that is a 30-fold more potent inhibitor of BCR-ABL1 activity and has

been approved for not only the treatment of newly diagnosed CP CML and CP CML in adult patients resistant or intolerant to prior therapy, but also stopping therapy in order to achieve treatment-free remission (TFR).

Frontline therapy

In the phase 3 randomized, open-label trial Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients (ENESTnd), nilotinib (300 mg twice daily or 400 mg twice daily) was compared with 400 mg/d of imatinib. CML patients on 300 mg or 400 mg twice daily of nilotinib had superior CCyR in 12 months compared with patients treated with imatinib 400 mg/d (80% and 78% vs 65%). The time to progression to AP or BP CML was better with the nilotinib-treated patients. Data from the 36-month follow-up showed the superiority of nilotinib 300 mg or 400 mg twice daily compared with 400 mg once daily of imatinib in terms of rates of MMR (73% and 70% vs 53%), MR 4.0 (50% and 44% vs 26%), rates of AP or BP CML progression (2 patients [0.7%] and 3 patients [1.1%] vs 12 patients [4.2%]), and incidence of mutations (11 patients in each nilotinib arm vs 21 in imatinib-treated patients). The most common mutations emerging with nilotinib use were T315I, Y253H, E255K, and F359V. The estimated 3-year OS was not statistically significantly different among the three groups (95%, 97%, and 94%), but the authors reported better OS for those treated with nilotinib compared with those treated with imatinib, if only CML-related deaths were considered (98.1% vs 98.5% vs 95.2%; HR, 0.35; $P=.0356$). By 5 years, more than one-half of all patients in each nilotinib arm (300 mg twice daily, 54%; 400 mg twice daily, 52%) achieved MR4.5 compared with 31% of patients in the imatinib arm. EMR rates were also higher in nilotinib-treated patients. A benefit of nilotinib was observed across all Sokal risk groups.

Second-line therapy (after imatinib resistance or intolerance)

Like dasatinib, nilotinib has also demonstrated significant clinical activity and an acceptable safety and tolerability profile in patients with imatinib-resistant or intolerant CP CML. Four-year follow-up from an international phase 2 study of CP CML in resistant/intolerant patients treated with nilotinib revealed that 59% achieved MCyR and 45% CCyR, and OS was estimated at 78%. Deeper responses at 3 and 6 months correlated with improved later outcomes, including OS. In an expanded-access, open-label study of 1,422 patients who failed prior imatinib, CCyR was attained in 34% of nilotinib-treated patients. In another study of patients in CCyR, but with detectable

BCR-ABL1 transcripts after more than 2 years on imatinib, patients randomized to nilotinib had higher rates of undetectable *BCR-ABL1* compared to those randomized to imatinib at 2 years (22.1% vs 8.7%, $P=.0087$); deeper responses (MR4.5) at 2 years were also more commonly observed in nilotinib-treated patients.

Toxicity

Unique toxicities associated with nilotinib use include hyperglycemia, hyperlipidemia, hyperbilirubinemia, and QT interval prolongation. Increasing recognition of vascular toxicities associated with nilotinib use is emerging, including cerebrovascular, cardiovascular, and peripheral arterial occlusive diseases, which have been reported in patients with or without cardiovascular risk factors. At 5-year follow-up of the ENESTnd study, ischemic heart disease, ischemic cerebrovascular events, and peripheral artery events were reported in 7.5%, 13.4%, and 2.1% of patients receiving nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib, respectively. It was also noted that the cumulative frequency of events increased over time on nilotinib treatment. As a consequence, nilotinib should be used with extreme caution in individuals with diabetes mellitus, cardiovascular disease, or metabolic syndrome. The mechanism of these events remains elusive, but recent studies suggest that vascular endothelial cells may play a role. Additionally, reports have suggested the increased risk

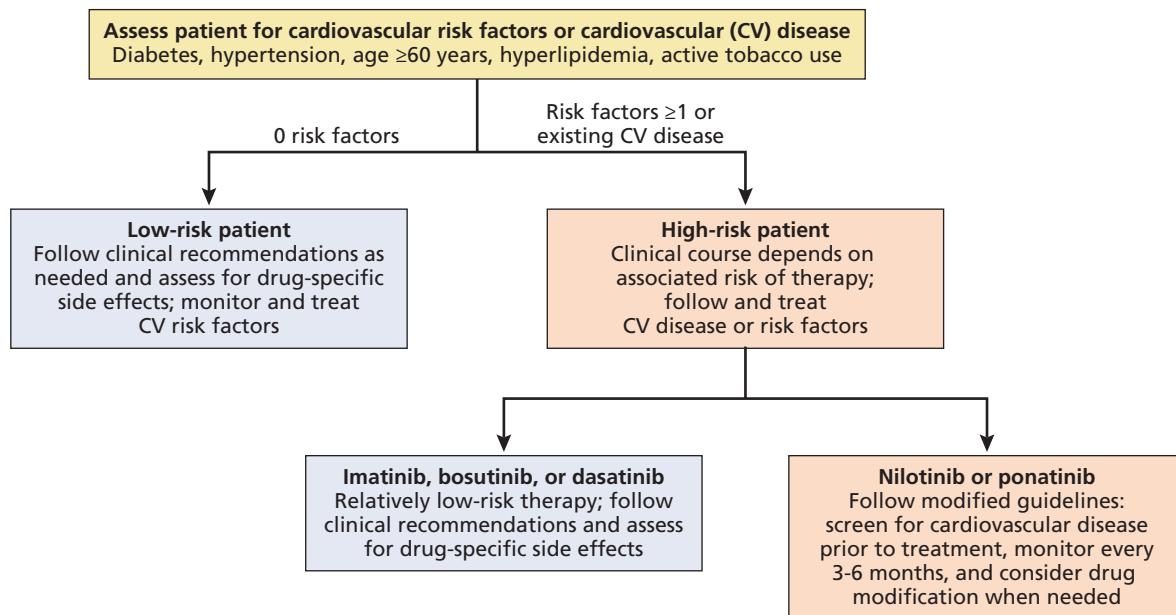
of hyperglycemia with nilotinib, as well as increasing body mass index and hyperlipidemia contribute to the increased risk of vascular events seen in nilotinib-treated individuals. Recent reviews have recommended increased monitoring of lipids and hemoglobin A1c at yearly to twice-yearly intervals in nilotinib-treated patients. An algorithm for determining the clinical management of low- and high-risk patients treated with nilotinib, as well as other TKIs, is shown in Figure 17-2 (discussed in detail in the CML education section from the ASH Annual Meeting, December 2017).

Bosutinib

Frontline therapy

Bosutinib, a dual Src/Abl kinase inhibitor, was very recently FDA-approved for the frontline treatment of CP CML based on results of the phase 3 randomized BFORE (Bosutinib Trial in First-Line Chronic Myelogenous Leukemia Treatment) trial, a follow-up study to the phase 3 Bosutinib Efficacy and Safety in Newly Diagnosed Chronic Myeloid Leukemia (BELA) trial, which compared bosutinib with imatinib in newly diagnosed CP CML. The BELA study did not achieve its primary endpoint, the rate of CCyR at 12 months, but did demonstrate a significant improvement in MMR rate at 12 months (41% vs 27%, bosutinib vs imatinib, respectively; $P=.001$). There were also fewer on-treatment transformations to AP or

Figure 17-2 An algorithmic approach to TKI treatment in low- and high-cardiovascular-risk patients. Cardiovascular risk factors include hypertension, cigarette and tobacco use, hyperlipidemia, and diabetes mellitus. Redrawn from Barber MC et al, *Hematology Am Soc Hematol Educ Program*. 2017;2017:110–114.



BP CML and fewer CML-related deaths with bosutinib. Because bosutinib given at 500 mg orally daily on the BELA study resulted in more frequent gastrointestinal and liver-related toxicities as compared to imatinib-treated patients, the BFORE study randomized 536 patients to bosutinib at 400 mg daily vs imatinib at 400 mg daily. The median dose intensity was 392 mg daily. At 12 months, MMR rates were significantly higher in bosutinib-treated patients as compared to imatinib-treated patients (47.2% vs 36.9%, respectively; $P=.02$) and were higher in patients with high Sokal risk scores (34.0% vs 16.7%, respectively). CCyR rates at 12 months were higher in patients receiving bosutinib as compared to imatinib (77.2% vs 66.4%, respectively). EMR (*BCR-ABL1* transcripts $\leq 10\%$ at 3 months) was achieved in a greater proportion of patients receiving bosutinib as compared to imatinib (75.2% vs 57.3%, respectively), and deeper molecular responses at 3, 6, 9, and 12 months were seen more frequently in bosutinib-treated patients. Similar to earlier studies of dasatinib and nilotinib, no statistically significant difference in OS or EFS was observed in patients receiving bosutinib as compared to imatinib. Four patients (1.6%) receiving bosutinib and six patients (2.5%) receiving imatinib experienced disease progression to AP or BP.

Second-line therapy (after imatinib resistance or intolerance)

Bosutinib was approved for the treatment of adult patients with CP, AP, or BP CML who are resistant or intolerant to imatinib, based on a single-arm, open-label multicenter study of CP, AP, and BP CML patients who received at least one prior TKI (either imatinib or imatinib followed by nilotinib or dasatinib). A total of 546 patients were enrolled, of which 73% were imatinib resistant and 27% were imatinib intolerant. Among 284 CP CML patients, cumulative MCyR and CCyR rates were 58% and 46%, respectively, by year 2 and 60% and 50%, respectively, by year 5. The cumulative MMR rate was 42%. Estimated OS was 91% at year 2 and 84% at year 5. The most frequent mutations newly emerging on bosutinib included T315I, V299L, and M244V. Specifically focusing on 119 patients receiving bosutinib in the third- or fourth-line setting after imatinib and nilotinib or dasatinib, or both, the cumulative 4-year MCyR rate was 40%, and 26% attained CCyR. At 4 years, the cumulative incidence of on-treatment progression and death was 24%.

Toxicity

Similar to other TKIs, bosutinib is also associated with myelosuppression, in particular thrombocytopenia. Unique toxicities associated with bosutinib use are primarily gas-

trointestinal, including diarrhea, nausea, vomiting, and transaminitis. In patients treated with second- or third-line bosutinib, diarrhea was common (86% and 83%, respectively), but the incidence of grade 3/4 diarrhea was low (10% and 9%, respectively). The most common grade 3/4 toxicity in resistant or intolerant patients was thrombocytopenia (25%). In the first-line BFORE study, the most common adverse events of all grades in bosutinib-treated patients were diarrhea (70.1%), nausea (35.1%), thrombocytopenia (35.1%), increased alanine aminotransferase (30.6%), and increased aspartate aminotransferase (22.8%). Similar to studies of bosutinib in intolerant or resistant patients, diarrhea was primarily grades 1 and 2, with only 7.8% of first-line bosutinib-treated patients having grade 3 diarrhea. Diarrhea symptoms responded to dose adjustments and improved in many patients over time. The incidence of pleural effusion, cardiovascular events, and peripheral vascular events was low.

Ponatinib

Ponatinib is approved to treat T315I-mutated CML and for the treatment of adult patients with CP, AP, or BP CML or Ph+ ALL for whom no other TKI therapy is indicated. The T315I mutation results in resistance to TKI therapy due to a threonine/isoleucine substitution resulting in steric inhibition, which prevents binding to and inhibition of the kinase domain. Options for patients with T315I mutations historically have included investigational agents, allogeneic SCT, or IFN therapy depending on the patient's age, comorbidity profile, and donor availability. A third-generation oral pan-BCR-ABL1 TKI, ponatinib, has shown significant activity in CML patients with T315I mutations or who are resistant to multiple TKIs. In the phase 2 Ponatinib Ph-positive acute lymphoblastic leukemia and CML Evaluation (PACE) trial, refractory CP, AP, and BP CML or Ph+ ALL patients resistant or intolerant to dasatinib or nilotinib, or with the T315I mutation, were treated with ponatinib (45 mg orally once daily). A total of 88% of the patients in the cohort had resistance to either dasatinib or nilotinib. Among 267 CP CML patients, 56% attained MCyR (51% with resistance or intolerance of dasatinib or nilotinib and 70% with the T315I mutation), 46% achieved CCyR (40% of those with resistance/intolerance and 66% with the T315I mutation, respectively), and 34% attained MMR (27% of those with resistance/intolerance and 56% with the T315I mutation, respectively). The median time to MCyR was rapid at 2.8 months, and the rate of sustained MCyR at 12 months was 91%. A recent meta-analysis of clinical trials of nilotinib, dasatinib, bosutinib, and ponatinib in the resistant/intolerant setting suggested that ponatinib may have increased

efficacy in CP CML after failure of second-generation tyrosine kinase inhibitors. Estimated probabilities of CCyR with treatment with another second-generation TKI after second-generation TKI failure ranged from 22% to 26% for second-generation TKIs, as compared with 50% to 60% for ponatinib. Based on these promising observations, the Evaluation of Ponatinib versus Imatinib in Chronic Myeloid Leukemia (EPIC) study randomized patients to receive oral ponatinib (45 mg) or imatinib (400 mg) once daily. Due to safety concerns emerging from phase 1 and 2 trials, this study was terminated early and did not meet its primary endpoint. Secondary analyses, however, demonstrated that more patients treated with ponatinib as compared to imatinib achieved MMR or MR4.5 at 3 months (31% vs 3% and 5% vs 0%, respectively).

Toxicity

Toxicities associated with ponatinib, primarily vascular, have limited its use. In the PACE study, the most common adverse events included thrombocytopenia, rash, dry skin, and abdominal pain. Updates to ponatinib labeling now report that arterial occlusive events have occurred in at least 35% of ponatinib-treated patients, including myocardial infarction, stroke, stenosis of large arterial vessels of the brain, and severe peripheral vascular disease, which have also occurred in younger individuals. Among 154 patients treated in the EPIC study, 7% of ponatinib-treated patients developed arterial occlusive events compared to 2% in the imatinib group, and the median time to onset was ~4 months. Because of these adverse events, ponatinib sales were briefly suspended in the United States. Ponatinib was formerly part of a risk evaluation and mitigation strategy in the United States with careful monitoring recommended. The mechanism of ponatinib vascular toxicity is not fully understood, but ponatinib treatment resulted in hypertension in 26% of patients in the PACE study. It is not yet clear if the thrombotic risk is dose dependent, and ongoing studies (eg, Optimising Ponatinib Treatment In CML (OPTIC) study) may answer this question. Consequently, the use of ponatinib requires a careful assessment of risk and benefit in individual patients, and further study is needed to delineate more clearly its use outside of T315I- mutated CML, as well as the appropriate dosing of ponatinib.

Selecting first-line TKI therapy in CP CML

As of 2018, four TKIs have been approved in the first-line setting. All are excellent choices, and there is no “right way” to select therapy. Overall, the goals of care are 1) to ensure response milestones are met, as this will ensure normal life span, 2) to optimize quality of life while taking daily medication, and 3) to minimize longer-term

potentially irreversible toxicities. To these goals is now added the possibility of achieving such deep and durable molecular responses that a trial of TKI discontinuation can be considered. Irrespective of this debate, the first goal is the most important. Longer follow-up of dasatinib and nilotinib clinical trials has not found statistically significant differences in OS or PFS for second-generation TKIs as compared to imatinib when used first line. Similar observations, with shorter follow-up, have been made for bosutinib. Nonetheless, there are benefits from the use of first-line dasatinib and nilotinib as compared to imatinib. These benefits include the development of fewer mutations conferring TKI resistance, decreased rates of progression to AP and BP, and more rapid achievement of MMR or MR4.5 at 5 and 6 years. However, as discussed earlier in this chapter, there are unique, and potentially life-altering, side effects associated with nilotinib and dasatinib. These include the increased risk for cerebrovascular, cardiovascular, and peripheral arterial events with nilotinib and pleural effusion and pulmonary hypertension with dasatinib. Consequently, a patient’s medical history and family history, together with their personal therapy goals, should be used to guide selection of first-line, second-generation TKIs. For example, avoiding nilotinib in patients with cardiovascular disease, diabetes mellitus, and/or uncontrolled hyperlipidemia is preferred. Avoiding dasatinib in patients with congestive heart failure or a history of pleural effusion and avoiding bosutinib in patients prone to diarrhea (eg., inflammatory bowel disease) are also reasonable strategies. Imatinib not only remains the most cost-effective choice, but also is the TKI with the longest safety track record. Imatinib is an excellent choice for many patients, including older patients with medical comorbidities. Scenarios in which to consider first-line, second-generation TKI use in CP CML patients include a high-risk Sokal score, although these patients also have poorer outcomes with second-generation TKIs, and the presence of additional chromosomal abnormalities at diagnosis. There is also an argument to consider second-generation TKI in younger female patients who may want to achieve deep responses quickly in order to plan treatment interruption for the purposes of family planning.

Molecular milestones and monitoring TKI therapy

Guidelines regarding milestones for response and recommendations for monitoring have been created by ELN and NCCN. These monitoring recommendations specify molecular monitoring at 3-month intervals and are generally based upon observations regarding outcomes from clinical trials. Early response or *BCR-ABL1* IS transcripts ≤10% at 3 months is recommended as a trigger to examine patient

adherence and assess resistance in patients not achieving this milestone. A study of 1,440 patients treated on the German CML Study IV observed that among the 28% of patients who did not achieve $\leq 10\%$ *BCR-ABL1*, OS after 5 years was poorer at 87% vs 94% for patients $>1\%$ but $\leq 10\%$ and as compared to 97% for patients $\leq 1\%$. Other studies have confirmed that early response at 3 months is associated with response, PFS, and OS in patients treated with second-generation TKIs. The benefit in improved PFS and OS for patients who achieve EMR is similar across studies and is ~10% to 15%. Although fewer patients achieve EMR on imatinib at 400 mg daily, the impact of achieving EMR on outcomes is similar for first- and second-generation TKIs. Not achieving EMR is likely a marker of poor biology, as more patients with high-risk Sokal scores do not achieve EMR. However, it may also reflect poor adherence. Although, the improved prognosis associated with EMR at 3 months is unquestioned, current treatment recommendations identify *BCR-ABL1* transcripts $>10\%$ as a warning rather than failure and suggest that response at 6 months can influence decisions to alter therapy. This recommendation is based not only upon observations from several studies, but also on a lack of evidence that very early change alters outcome. A study of 320 imatinib-treated patients demonstrated that patients with *BCR-ABL1* transcripts $>10\%$ at 3 months but $<1\%$ at 6 months had no significant difference in PFS as compared to patients achieving *BCR-ABL1* transcripts $<10\%$ at 3 months. The Australian group reported similar observations among 528 imatinib-treated patients and identified that only the group of patients with *BCR-*

ABL1 transcripts $>10\%$ at 6 months had poorer PFS and OS. Similar observations at 6 months have been made for patients treated with frontline nilotinib and dasatinib. It is not clear how early treatment interruptions to manage side effects have influenced these analyses, such that the 3-month milestone might be too early to definitively assess efficacy. Additional milestones are based upon molecular and cytogenetic data demonstrating the association between PFS, EFS, and OS and response at particular time points during therapy. *BCR-ABL1* transcripts of $<1\%$ are roughly equivalent to CCyR, and most physicians now use molecular testing rather than the more invasive karyotyping. For the IRIS trial at 6-year follow-up, the EFS rate was 59%, 85%, and 91% for patients with no cytogenetic response, MCyR, or CCyR at 6 months, respectively, and other studies have demonstrated improved OS in patients with CCyR at 6 or 12 months. MMR is associated with improved EFS and PFS and decreases the risk for loss of response, but the time at which MMR should be achieved is more controversial. Deeper molecular response appears to limit progression further. Among patients achieving MR4.5 on the German CML Study IV receiving imatinib or imatinib combination therapies, there were no progressions among patients achieving MR4.5, as compared to 1, 9, and 13 events in patients whose deepest responses were MR4.0, MMR, and CCyR, respectively.

Recommendations from ELN are shown in Table 17-3; NCCN also characterizes responses as optimal (green), warning (yellow), or failure (red) in their guidelines (Figure 17-3). No EMR at 3 months is a warning, and no EMR

Table 17-3 Expected milestones and response to first-line TKI therapy (EMSO provisional adaptation of ELN 2013 recommendations)

Time	Optimal	Warning	Failure
Diagnosis		High-risk score CML, major route ACA	
3 months	MCyR (Ph-positive metaphases $\leq 35\%$) and/or <i>BCR-ABL1</i> $\leq 10\%$	Less than MCyR (Ph-positive metaphases 36% to 95%) and/or <i>BCR-ABL1</i> $>10\%$	No CHR and/or Ph-positive metaphases $>95\%$
6 months	CCyR (Ph-positive metaphases 0%) and/or <i>BCR-ABL1</i> $<1\%$	Less than CCyR (Ph-positive metaphases 1% to 35%) and/or <i>BCR-ABL1</i> 1% to 10%	Less than MCyR (Ph-positive metaphases $>35\%$) and/or <i>BCR-ABL1</i> $>10\%$
12 months	MMR (<i>BCR-ABL1</i> $\leq 0.1\%$)	Less than MMR (<i>BCR-ABL1</i> 0.1% to 1%)	Less than CCyR (Ph-positive metaphases $>0\%$) and/or <i>BCR-ABL1</i> $>1\%$
>18 months	BCR-ABL $<0.01\%$ in patients who are candidates for TFR	Less than MMR (<i>BCR-ABL1</i> 0.1% to 1%)	
At any time	<i>BCR-ABL1</i> $\leq 0.1\%$		Loss or CHR, CCyR, or MMR, or acquisition ABL TKD mutations or additional cytogenetic abnormalities

Optimal responses correlate with favorable long-term outcomes, and no treatment change recommended. Warning suggests a need for more frequent monitoring to identify any need to change therapeutic strategies. Failure requires a change in therapeutic strategy.

Response milestones				
BCR-ABL1 (IS)	3 months	6 months	12 months	>12 months
>10%	Yellow	Red		
1% – 10%	Green		Yellow	Red
0.1% – <1%	Green			Yellow
<0.1%	Green			

Clinical considerations		Second-line and subsequent treatment options
Red	Evaluate patient compliance and drug interactions Mutational analysis	Switch to alternate TKI and evaluate for HCT
Yellow	Evaluate patient compliance and drug interactions Mutational analysis	Switch to alternate TKI or continue same TKI or dose escalation of imatinib (to max of 800 mg) and evaluate for HCT
Green	Monitor response and side effects	Continue same TKI

Figure 17-3 Expected milestones and response to first-line TKI therapy as recommended by NCCN. Redrawn and adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Chronic Myeloid Leukemia V.4.2018. © 2018 National Comprehensive Cancer Network, Inc. All rights reserved. The NCCN Guidelines® and illustrations herein may not be reproduced in any form for any purpose without the express written permission of NCCN. To view the most recent and complete version of the NCCN Guidelines, go online to [NCCN.org](https://nccn.org).

at later time points is a failure. *BCR-ABL1* transcripts between 1% and 10% at 12 months is a warning and after 12 months is a failure. *BCR-ABL1* transcripts between 0.1% and 1% after 12 months is a warning. *BCR-ABL1* transcripts should be measured at diagnosis to establish a baseline and every 3 months after initiating therapy. NCCN suggests that once *BCR-ABL1* transcripts of 0.1% to 1% IS are achieved, *BCR-ABL1* transcripts should be monitored every 3 months for 2 years and then every 3 to 6 months thereafter. If transcripts rise by 1 log or more in the setting of MMR, *BCR-ABL1* transcripts should be measured at 1- to 3-month intervals.

As discussed in the section entitled “Pathobiology,” mutations in the ABL TKD are a common cause of TKI resistance. Mutations, identified by sequencing, should be assessed if *BCR-ABL1* transcripts are >10% at 3 or 6 months or there is failure to meet other response milestones, loss of cytogenetic or hematologic response occurs, a 1-log or greater increase in *BCR-ABL1* transcript levels together with a loss of MMR occurs, or disease progression occurs. Recommendations for options for next-line TKI therapy, based on the most common mutations detected, are shown in Table 17-4. Patients should also be assessed for adherence (see below). A bone marrow examination should be considered if response milestones are not met and if hematologic or cytogenetic response is lost. An

Table 17-4 Recommendations for selecting next-line TKI therapy after TKD mutation detection

Mutation	Treatment recommendation
Y253H, E255K/V, or F359V/C/I	Dasatinib
F317L/V/I/C, T315A, or V299L	Nilotinib
E255K/V, F317L/V/I/C, F359V/C/I, T315A, or Y253H	Bosutinib
T315I	Ponatinib, omacetaxine, allogeneic SCT

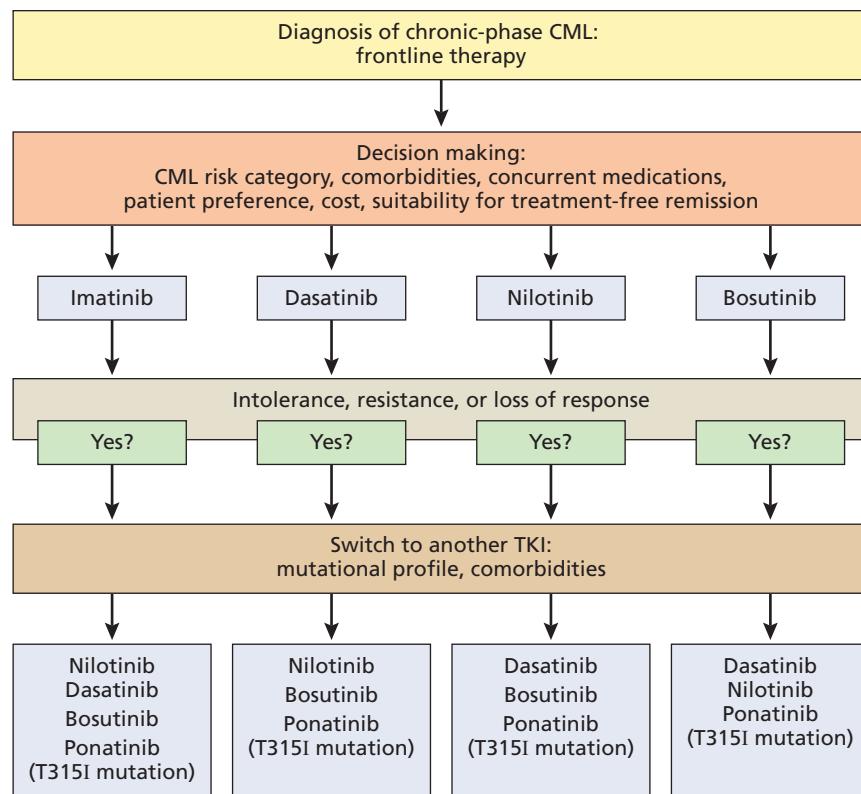
The most common mutations detected are shown. Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Chronic Myeloid Leukemia V.4.2018. © 2018 National Comprehensive Cancer Network, Inc. All rights reserved. The NCCN Guidelines® and illustrations herein may not be reproduced in any form for any purpose without the express written permission of NCCN. To view the most recent and complete version of the NCCN Guidelines, go online to [NCCN.org](https://nccn.org).

overall approach to first- and second-line CML treatment is shown in Figure 17-4.

Adherence and treatment failure

Another important, but difficult to quantify, contributor to treatment failure is poor therapy adherence. Monitoring recommendations suggest that adherence be assessed whenever response milestones are not met. Although it is difficult to compare studies head to head given the

Figure 17-4 A proposed algorithm for CML treatment. Decision-making for CML treatment in the first- and second-line is shown. Patients with disease that is resistant to primary treatment with imatinib should be treated with dasatinib, nilotinib, or bosutinib in the second-line setting based upon mutational screening results and comorbidity assessment. Patients with disease that is resistant to primary treatment with dasatinib, nilotinib, or bosutinib can be treated with an alternate TKI (other than imatinib) in the second-line setting. Ponatinib is a treatment option for patients with a T315I mutation and, as indicated on the label, for patients for whom no other TKI is indicated. However, caution should be exercised when selecting next-line therapy in patients failing first-line, second-generation TKI therapy if they are fully adherent to therapy, but are resistant without evidence of TKD mutations. Studies support that this is a group of patients who are at increased risk for failing a second-line, second-generation TKI and earlier consideration for ponatinib may be warranted. Omacetaxine is a treatment option for patients with disease that is resistant and/or intolerant to 2 or more TKIs.



differences in assessment of adherence (eg., chart review vs pill counts vs electronic devices to measure bottle opening vs review of health care databases), the rates of nonadherence at 25% to 35% are similar in many of these studies. Definitions for nonadherence on imatinib included the use of ≤85% or 90% of prescribed drug. The ADAGIO study examined adherence in 169 patients in Belgium and observed that approximately one-third of patients were nonadherent and only 14.2% of patients were 100% adherent with prescribed imatinib. Nonadherence was associated with poorer cytogenetic response. In another study of 87 patients treated at Hammersmith Hospital in London, patients with adherence rates of ≤85% had an increased probability of losing CCyR (26.8% vs 1.5%).

The ADAGIO study identified several factors that adversely impacted adherence, including age, living alone, dose of imatinib, male sex, length of time from diagnosis to treatment, and length of imatinib treatment. Factors that positively influenced adherence included increased knowledge about CML disease and treatment, at least a secondary education, and taking other medications chronically. A Hammersmith Hospital study of patient adherence further explored issues and behavior contributing to nonadherence. The most common reason for nonintentional nonadherence was forgetfulness, while the most common

reason for intentional nonadherence was to minimize side effects. Notably, many patients did not think missing doses would significantly impact their response, and patients relied upon their treating health care teams to comment on the impact of nonadherence on treatment responses. These observations suggest that a proactive approach may improve adherence. Suggestions include nursing or pharmacist phone calls or visits to assess for adverse effects, dispensing pill boxes to assist taking pills on schedule, recommendations to link TKI use to a regular scheduled daily activity, cell phone alerts, and taking particular care to discuss these issues with patients who have risk factors for nonadherence. Of note, an early study reported that short *BCR-ABL1* transcript doubling time could distinguish nonadherence from resistance and may assist physicians in recognizing nonadherent patients.

Discontinuation of TKI therapy and dose de-escalation in responding patients

Stopping TKI therapy with the goal of TFR is now part of current treatment recommendations and guidelines. Despite early concerns that imatinib and other TKIs do not target CML stem cells and discontinuation would be risky, a considerable body of work over the past decade supports the safety of this intervention. The initial studies in

France (Stop Imatinib, STIM) and Australia (TWISTER) enrolled adult patients who had been treated with imatinib and had achieved deep and durable responses, defined as a >5-log reduction in *BCR-ABL1* transcript levels, for >2 years. TKI therapy was restarted at the time of molecular recurrence. Of 100 patients in the STIM study with a median follow-up of 77 months (range, 9 to 95 months), 38% remained in complete molecular response at 60 months. Molecular recurrence was most frequent within 6 months of stopping imatinib therapy. Treatment was restarted in 57 of 61 patients with molecular recurrence, and 55 patients achieved a second complete molecular response at a median time of 4 months (range, 1 to 16 months). TWISTER reported similar TFR rates (47%) and also observed that molecular relapse, when it occurred, occurred early. Reassuringly all patients regained deep molecular responses upon restarting therapy. Subsequent studies, such as A-STIM, also investigated the possibility of stopping imatinib in patients with less deep molecular responses and more clearly defined when therapy should be restarted, namely at the time of loss of MMR. Notably, fluctuations of *BCR-ABL1* transcript levels below the MMR threshold were observed in 31% of patients after discontinuation.

A meta-analysis of 15 stopping studies, containing 509 patients, showed cumulative molecular relapse rates of 30%, 41%, 44%, and 50% at 3, 6, 12, and 24 months. Although most relapses occurred early (55% within 3 and 80% within 6 months), late relapses were observed up to 22 months post discontinuation. ELN has recently conducted a large multicenter study of treatment discontinuation in patients who have received TKI for at least 3 years and have achieved and sustained MR4.0 for at least 1 year (EURO-SKI). The results show similar relapse-free survivals as earlier studies. Factors predictive of successful discontinuation were duration of imatinib treatment greater than a median of 5.8 years and duration of deep molecular response of 3.1 years or longer.

Two studies have examined stopping dasatinib or nilotinib after imatinib intolerance or resistance. Results are similar to those of stopping imatinib, but both studies reported higher rates of molecular recurrence in patients with resistance to imatinib. To date, only one study has reported the outcome of stopping a second-generation TKI given from diagnosis, which has led to licensing approval for the discontinuation of nilotinib for the purposes of TFR. ENESTfreedom enrolled 215 patients who had achieved MR4.5 and had received a minimum of 2 years treatment with nilotinib and treated them with standard-dose nilotinib for a further year. At that point, 190 patients discontinued nilotinib, and 48 weeks later, 98 of 190

patients (51.6%) remained in MMR without treatment re-initiation. Other strategies to improve the numbers of patients eligible for TFR include the possibility of a second attempt at discontinuation in patients who experienced recurrence after their first attempt and switching imatinib-treated patients who have not achieved the depth of response required for consideration of discontinuation to a second-generation TKI to see if the response can be improved.

Recommendations for stopping TKI outside of the context of clinical trials have been endorsed by organizations such as NCCN based on published recommendations for management. Access to high-quality RT-QPCR monitoring and monthly estimations of *BCR-ABL1* transcript levels, particularly in the first 6 to 12 months, is mandatory. Patients should have an easily quantifiable transcript type amenable to standardized technology, have generally achieved optimal responses according to ELN, have been treated for at least 3 years, and have deep molecular response of MR4.0 or better for at least 2 years. An approach proposed by Australian investigators is shown in Figure 17.5. Approximately 25% of patients experience a “withdrawal syndrome” on stopping TKI, which is manifested by musculoskeletal pain occurring 1 to 6 weeks after discontinuation and/or generalized pruritus. The pain can resemble polymyalgia rheumatica or cause arthralgia, particularly of hips, shoulders, hands, and feet. It usually resolves spontaneously, although this might take many weeks and in some cases months.

An alternative approach was taken in the UK DESTINY study, which explored the benefit of an initial 12-month period of a 50% dose reduction from standard doses of imatinib, nilotinib, or dasatinib. Eligibility criteria required a minimum treatment period of 3 years and included patients in MMR, in addition to those in MR4.0 or deeper for at least 12 months. The trigger for restarting TKI was loss of MMR. After 1 year of dose reduction, recurrence was significantly lower in the MR4.0 cohort (3 [2%; 90%CI, 0.2 to 4.8] of 121 evaluable patients) than in the MMR cohort (9 [19%; 90%CI, 9.5 to 28.0] of 48 evaluable patients; HR, 0.12, 90%CI, 0.04 to 0.37; $P=.0007$).

TKI therapy in accelerated and blast-phase CML

In a published phase 2 study of imatinib-treated patients with AP CML, CHR, MCyR, and CCyR occurred in 53%, 24%, and 17% of patients, respectively. Survival and progression-free survival rates at 12 months were optimal among patients receiving 600 mg/d (78% and 44%,

Criterion	Green	Yellow	Red
Sokal score at diagnosis	Non-high	High	
BCR-ABL1 transcript at diagnosis	Typical e13a2 or e14a2	Atypical, but can be accurately quantified	Not quantifiable
CML history	CP only	Resistance or TKD mutation	Prior AP or BP
Response to first-line TKI therapy	Optimal	Warning	Failure
Duration of all TKI therapy	>8 years	3–8 years	<3 years
Depth of molecular response	MR 4.5	MR 4.0	Not in MR 4.0
Duration of deep molecular response monitored in a standardized laboratory	>2 years	1–2 years	<1 year

Figure 17-5 An approach for considering which patients are candidates for TKI cessation. The National Comprehensive Cancer Network (NCCN) CML Panel has provided guidance on the selection of patients who are appropriate for a trial of TKI cessation. These minimum criteria include that patients have 1) CP CML with no history of AP or BP, 2) a quantifiable *BCR-ABL1* transcript, 3) have been on an approved TKI for a minimum of three years, and have 4) stable deep molecular response of $\leq 0.01\%$ on at least four tests (at least 3 months apart) for at least 2 years. Additional factors have been included herein, such as duration of TKI therapy and the presence of TKI resistance, which have been described to impact TFR in clinical studies. All green is a strong recommendation to consider TKI withdrawal; for any yellow, consider caution (e.g. withdrawal in high-priority settings such as significant adverse events or planned pregnancy); for any red, TKI withdrawal is not recommended. Redrawn and adapted from Hughes TP and Ross DM, *Blood*. 2016;128(1):17–23.

respectively). For *de novo* AP patients, defined using ELN criteria, subsets of patients who may respond well to first-line imatinib have been identified. Patients with AP, as defined solely by blast percentage, as compared to patients with additional cytogenetic aberrations and elevated blast percentage, had improved rates of major and complete cytogenetic response (94% vs 40% and 81% vs 30%, respectively) and failure-free survival (87.5% vs 15%, respectively). For patients with poor risk features, imatinib treatment may serve as a bridge to allogeneic SCT.

Imatinib can transiently control CML blast phase in a proportion of patients and serves as a bridge to SCT in patients who are candidates for SCT. Both lymphoid and myeloid phenotypes respond, and optimal results are achieved with a dose of 600 mg/d. Imatinib induced overall hematologic responses in ~50% of study subjects, 8% to 21% achieved CHRs, and ~30% achieved stable or sustained hematologic responses (lasting ~4 weeks). MCyRs occurred in 16% of patients, and CCyRs occurred in 7% of patients. The median overall survival for patients who achieved a sustained hematologic response was 19 months. Myelosuppression was common, and nonhematologic toxicities were mild to moderate.

Dasatinib, at a dose of 140 mg/d, led to CHR, MCyR, and CCyR in 45%, 39%, and 32% of patients with AP CML, respectively. Responses were achieved in imatinib-resistant and intolerant patients. The 12-month PFS and OS rates were 66% and 82%, respectively. In another study,

a subgroup of patients with AP CML randomized to 140 mg once daily or 70 mg twice daily experienced comparable rates of major hematologic response (MHR; 66% vs 68%) and MCyR (39% vs 43%), but once-daily dosing was associated with a more favorable safety profile. Two-year follow-up from a study of patients with BP CML treated with either 140 mg daily or 70 mg twice daily suggested that once-daily dosing had comparable efficacy and better tolerability. In those with myeloid BP CML treated with once-daily dasatinib, the MHR was 28%, MCyR was 25%, and OS at 24 months was 24%. For those with lymphoid BP CML, corresponding rates were 42%, 50%, and 21%, respectively. Dasatinib is approved for AP and myeloid or lymphoid BP CML with resistance or intolerance to other therapy.

With 2 years of follow-up, nilotinib, at a dose of 400 mg orally twice daily in patients with AP CML, led to CHR, MCyR, CCyR, and MMR in 31%, 32%, 21%, and 11% of patients, respectively. The 24-month overall survival rate was 70%. Nilotinib is approved for use in AP CML with resistance or intolerance to other therapy.

Bosutinib has been approved for AP and BP CML with resistance or intolerance to prior therapy. Updates of advanced-phase patients with ≥ 4 years of follow-up demonstrated that among AP and BP patients, 57% and 28%, respectively, attained or maintained overall hematologic response and that 40% and 37%, respectively, attained or maintained MCyR. Lastly, ponatinib is an option for those

with advanced disease and intolerance/resistance to prior therapy. Among 82 patients with AP CML, 55% achieved MHR, 39% MCyR, 24% CCyR, and 16% achieved MMR. Among patients with BP CML, 31% achieved MHR, 23% MCyR, and 18% CCyR.

Overall, outcomes for BP CML remain dismal even in the era of TKIs, although a subset of BP CML patients, as defined by WHO criteria with blast percentages of 20% to 29%, may have outcomes more similar to AP patients. A recent retrospective review of 477 BP patients attempted to identify characteristics or prognostic factors associated with outcomes. Among this group, 72% had received prior TKI therapy before progression. Median OS in this group was 12 months, and median failure-free survival was 5 months. As initial therapy for BP, 35% received TKI alone, 46% TKI with chemotherapy, and 19% non-TKI therapy. Factors that predicted for increased risk of death in multivariate analysis included myeloid immunophenotype, prior TKI, age ≥ 58 years, lactate dehydrogenase level $\geq 1,227$ IU/L, platelet count $< 102,000/\mu\text{L}$, no history of stem cell transplantation, transition to BP from CP/AP, and the presence of chromosome 15 aberrations. Additionally, as reported in other studies, achievement of major hematologic response and/or CCyR to first-line treatment was predictive of improved OS. This study also suggested that combination chemotherapy with TKI followed by SCT conferred the best outcome. Although in lymphoid BP chemotherapy with TKI can be more effective, whether combination chemotherapy and TKI results in improved outcomes in myeloid BP is unclear.

Additional treatment strategies

Omacetaxine

Omacetaxine, a protein translation inhibitor formerly known as homoharringtonine, was approved by the FDA in 2012 for patients with CP or AP CML and with resistance or intolerance to at least two TKIs. This approval was based on a trial with MCyR rates of 20% in CP CML and MHR of 27% in AP CML. The final analysis, with 24 months follow-up, reported an MCyR and median OS of 18% and 40.3 months, respectively, in those with CP CML; 14% of patients with AP CML achieved MHR, for a median of 4.7 months. The most common toxicities were hematological, with at least grade 3 adverse events in 79% and 73% of CP and AP CML patients, respectively.

Asciminib (ABL001)

Asciminib is a targeted ABL inhibitor that binds to the myristoyl pocket of BCR-ABL instead of the catalytic pocket and induces the formation of an inactive kinase

conformation. In phase 1 data presented in abstract form, asciminib appeared to be well tolerated and resulted in durable activity in heavily pretreated CML patients, including CCyR and MMR. Mutations in the myristoyl pocket were rare but detectable in patients with relapse. Based on this activity, an ongoing phase 3 study of CP CML patients is randomizing patients to asciminib or bosutinib in resistant patients previously treated with two or more TKIs.

Other targeted approaches

A number of other treatment strategies for CML are under evaluation. These include approaches to eradicate CML stem cells using combination approaches with TKI and other agents such as JAK2 inhibitors (ruxolitinib) or PPAR- γ activators. Recent *in vitro* and *in vivo* work suggests that the combination of MDM2 and BET inhibitors may be used to upregulate p53-induced apoptosis and downregulate MYC to eradicate CML leukemia stem cells. These promising strategies, and others, are beyond the scope of this review, but are outlined in the American Society of Hematology Annual Meeting Education series on CML in 2017.

Stem cell transplantation

With the development of TKIs, rates of allogeneic SCT have dramatically declined for CP CML patients. Currently, allogeneic SCT is typically reserved for those who fail available TKIs and those with advanced-phase disease. For CP CML patients, typing can be considered at the time of failure or intolerance of second-line therapy when initiating third-line therapy. However, there may be scenarios when SCT may be considered at an earlier time. These may include, for example, pediatric or young adult patients who are adherent to therapy and fail first-line therapy with a second-generation TKI and do not have mutations associated with resistance that are amenable to treatment with an alternative TKI, or patients with T315I mutations. For *de novo* AP patients, SCT should be considered at diagnosis, but transition to SCT may depend on risky features at diagnosis (eg., ACAs and elevated blast count) and response to first-line TKI therapy. The phase of disease has a significant impact on transplant outcome, as is highlighted by recent data from the Center for International Blood and Marrow Transplant Research (CIBMTR). Outcomes are best in CP and are poor in BP, and consequently, timing of SCT before disease progression is critical. Data from CIBMTR are available for 2,015 HLA-matched sibling donor transplants spanning 2005 to 2015. Three-year probability of survival for CP

(N=1,611) was 66% ± 1%, for AP (N=249) was 51% ± 4%, and for BP (N=155) was 29% ± 4%. For CP patients, prior use of TKIs does not appear to influence transplant outcomes. For BP patients, inducing second CP yields outcomes comparable to AP transplant outcomes (ie, 20% to 40% long-term, disease-free survival). Second CP can be induced by TKI therapy or by TKI therapy in combination with induction chemotherapy similar to that used for acute leukemia. For children and young adults, a retrospective study of 449 patients found 5-year OS and leukemia-free survival after SCT of 76% and 57% in those aged <18 years and 74% and 60% in the 18- to 29-year-old group, respectively. In multivariate analysis, age and pre-SCT TKI use did not impact outcomes and older age was associated with an increased incidence of chronic graft-versus-host disease (cGVHD).

Across all ages, the incidence of acute GVHD ranges from 8% to 63%, with severe and fatal GVHD affecting up to 20% and 13% of patients, respectively. The use of alternative donors is expanding access to those in need of transplantation without a matched donor. Given the age of most CML patients and the fact that CML cells are highly susceptible to the graft-versus-leukemia (GVL) effect of an allograft, the use of reduced-intensity conditioning (RIC) regimens is common and has resulted in improved outcomes. The overall leukemia relapse rate after matched-unrelated donor SCT is somewhat lower than after matched-related transplants, suggesting that minor antigen disparity enhances a GVL effect. In addition, relapse rates are higher after transplantation with T-cell-depleted stem cells compared with unmanipulated stem cells, implicating that donor graft immune function is important in clearing residual disease. In a recent study of 306 CML patients predominantly treated with imatinib before SCT and receiving peripheral blood grafts and RIC, outcomes were examined for patients aged 40 to 49 years, 50 to 59 years, and 60 years or older. Unrelated donor RIC SCT was more common in older patients. Three-year OS was 54%, 52%, and 41%, respectively, and 3-year disease-free survival was 35%, 32%, and 16%, respectively. Three-year rates of chronic GVHD were 58%, 51%, and 43%, respectively, and 1-year treatment-related mortality was similar across age groups and was 18%, 20%, and 13%, respectively.

The potency of the GVL effect is further illustrated by the success of donor lymphocyte infusion (DLI) for relapsed disease after SCT. CML is the disease that responds best to DLI, although it is more effective in the treatment of CP relapse as compared to advanced-phase relapse. DLI induces remission in 54% to 93% of patients with early hematologic or cytogenetic relapse after allograft-

ing. TKI therapy is often effective in the setting of post-transplant relapse and can be used when GVHD is present and DLI is not an option. A review of 12 CP CML cases receiving imatinib after relapse reported that all patients achieved CCyR, and all but one had undetectable *BCR-ABL1* transcripts after 3 to 27 months of therapy (median, 9 months). Outcomes for patients with advanced-phase disease at relapse are not as good. A recent study of 14 advanced-phase patients reported CCyR rates of 71% and undetectable *BCR-ABL1* transcripts in 57%, either with imatinib or dasatinib treatment alone or in combination with donor lymphocyte infusion (DLI). The achievement of undetectable transcripts was very strongly associated with OS. Accordingly, molecular monitoring in the post-transplant setting is important to identify those at higher risk for relapse. Lastly, given the higher risk for relapse for advanced-phase patients after SCT, TKI therapy is often recommended for at least 1 year after SCT in these patients.

Parenting children

A small, but important, proportion of female patients are diagnosed with CML during the early stages of pregnancy as a result of routine laboratory tests. This difficult scenario must be handled sensitively. If presentation is in CP, there is no medical reason to terminate the pregnancy. However, TKIs are contraindicated in pregnancy because of an increased risk of congenital malformations, in particular omphalocele, and should not be used, particularly in the first and second trimesters. If the total white blood cell count is relatively low, some patients may complete the pregnancy without treatments. Others might be suitable for management by leukapheresis and/or INF. For the woman presenting with advanced-phase disease, the balance of risk for mother and child should be frankly discussed with the patient and partner.

The more frequent situation is the patient who wishes to parent a child after the diagnosis is established. For male patients treated with imatinib or dasatinib, there is a body of data to suggest that there is no increased risk to the mother during the pregnancy or to the infant. There are few data reporting pregnancies where the father is on nilotinib, bosutinib, or ponatinib, but there is no immediate reason to think that the risk would be different from that of imatinib and dasatinib.

For women on TKIs, treatment should be discontinued before conception. Ideally, the criteria for stopping TKI should be identical to that of trials for TFR, as approximately one-half the women are able to discontinue

indefinitely. The remainder experience molecular recurrence within the first 6 months, but if they have conceived within that period, there is a high probability that they will reach the end of the pregnancy before they require treatment. In “real life,” many women have not had prolonged deep responses at the time of considering motherhood. These situations should be handled individually, but possibilities include consideration of assisted conception techniques to minimize the time off treatment. If treatment is required during the pregnancy, then leukapheresis and interferon can be used early in the pregnancy. Because the teratogenic effect of imatinib appears to be during organogenesis, it is possible that it is safe in later pregnancy. However, a report of hydrops fetalis in a patient who received dasatinib in the second trimester underlines the need for caution. An important question, in particular for children, adolescents, and young adults on TKI therapy, is whether long-term TKI use impacts fertility. Case reports of primary ovarian insufficiency and oligospermia have been published, but, to date, very few data exist to inform decision making. These observations highlight the need for larger studies in younger patients examining TKI cessation, dose reduction, and/or intermittent TKI use.

KEY POINTS

- CML is a pluripotent hematopoietic stem cell neoplasm characterized by the *BCR-ABL1* fusion gene, which is derived from a balanced translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11), also known as the Ph chromosome.
- Typical blood findings include a left-shifted leukocytosis, with basophilia and often thrombocytosis.
- Prognosis has been remarkably improved by the development of TKIs and is dependent on the phase at presentation (CP, AP, or BP) and depth of response to therapy.
- There are now five TKIs available for use in CP-CML. Imatinib, dasatinib, nilotinib, and bosutinib are frontline options for CP CML. Dasatinib, nilotinib, bosutinib, and ponatinib can be used in those with intolerance or resistance to prior TKI therapy.
- Meeting treatment milestones strongly influences prognosis and identifies those with resistance or loss of response, who require a switch to another TKI. Consensus guidelines are available to direct appropriate assessments during months 3, 6, and 12 and beyond and aid in management decisions.
- TKI cessation and TKI dose reductions are possible in some patients. However, patients must be carefully selected and closely monitored.

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Myeloproliferative neoplasms

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The online version of this chapter contains an educational multimedia component on JAK-STAT activation by recurrent mutations leading to myeloproliferation.

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Dr. Stein: membership on scientific advisory board: Incyte. Dr. Gerdts: membership on advisory board: Incyte.

Off-label drug use: Interferon for MPNs.

Introduction

The myeloproliferative neoplasms (MPNs) are a phenotypically diverse group of stem cell–derived clonal disorders characterized by myeloid proliferation. MPNs share several clinical and laboratory features, including a pronounced symptom burden that impacts quality of life; a thrombotic tendency; frequent organomegaly (hepatomegaly or splenomegaly); and a potential to undergo a progression that terminates in marrow failure caused by fibrosis or in transformation to “blast phase.” In recognition of these shared clinical, laboratory, and histological features, William Dameshek first used the term *myeloproliferative disorders* in 1951 to classify essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). Dameshek also speculated on a shared pathogenesis, due to the presence of a “myelostimulatory factor.”

In 2005, Dameshek’s hypothesis was confirmed after the discovery of the activating Janus kinase (*JAK2*) V617F mutation. This discovery ushered in a new era of discovery and understanding. The molecular genetic landscape in Dameshek’s classical MPNs has since become well characterized. Activating point mutations of *JAK2* are observed in almost all patients with PV and in a significant proportion of patients with ET and PMF. Calreticulin (*CALR*) mutations are observed in substantial proportions of *JAK2*V617F-negative ET and Myelofibrosis (MF) patients. Somatic activating *JAK2* exon 12 mutations and myeloproliferative leukemia (*MPL*) mutations are less frequently identified mutations in *JAK2* V617F-negative PV and ET/MF patients, respectively. Regardless of the type, these driver mutations all activate the JAK-STAT signaling pathway.

Other MPNs have been found to harbor consistent molecular genetic abnormalities as well. Mutations in *CSF3R*, which encode the granulocyte colony-stimulating factor receptor, have been described in most patients with chronic neutrophilic leukemia (CNL). In addition, systemic mastocytosis (SM), now considered distinct from the classical MPNs, is frequently associated with somatic mutations in *KIT* (eg, *KIT* D816V). Finally, myeloid neoplasms with eosinophilia are characterized by rearrangements involving platelet-derived or fibroblast growth factor receptors (*PDGFRA*, *PDGFRB*, *FGFR1*), or *JAK2* point mutations or translocations.

As a result of these discoveries, Dameshek’s myeloproliferative disorders are now classified by the World Health Organization (WHO) as clonal, neoplastic

Table 18-1 2016 WHO classification of MPNs and related disorders

MPNs
CML, <i>BCR-ABL1</i> positive
CNL
PV
PMF
PMF, prefibrotic/early stage
PMF, overt fibrotic stage
ET
CEL-NOS
MPN, unclassifiable
Systemic mastocytosis
Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of <i>PDGFRA</i> , <i>PDGFRB</i> , or <i>FGFR1</i> , or with <i>PCM1-JAK2</i>
Myeloid/lymphoid neoplasms with <i>PDGFRA</i> rearrangement
Myeloid/lymphoid neoplasms with <i>PDGFRB</i> rearrangement
Myeloid/lymphoid neoplasms with <i>FGFR1</i> rearrangement
Provisional entity: myeloid/lymphoid neoplasms with <i>PCM1-JAK2</i>

entities (MPNs; Table 18-1). Without question, these molecular genetic abnormalities aid the clinician's diagnostic capabilities, prognostic assessments, and therapeutic choices. However, these mutations do not replace, but rather complement, clinical, laboratory, and histological findings that allow for diagnosis of the distinct MPN subtype. Establishing a diagnosis with accuracy can be challenging given that the MPNs discussed here can mimic one another. Yet, this is paramount to management given prognostic and therapeutic implications. In this chapter, the impact from driver mutations as well as the diagnosis, clinical features, treatment, and prognosis of classical and atypical MPNs are reviewed.

Driver mutations

JAK2 mutations

A watershed moment in the understanding of the MPNs arose in 2005 when *JAK2* V617F was discovered in patients with ET, PV, and MF (Table 18-2). *JAK2* is an intracellular signaling molecule coupled to several cell surface hematopoietic growth factor receptors that lack intrinsic kinase domains, including the erythropoietin (EPO) receptor, GCSF receptor, and the thrombopoietin receptor, c-MPL. The *JAK2* V617F point mutation is thought to result in loss of the pseudokinase domain's (JH2) inhibitory control of the kinase domain (JH1) (see video on *JAK2*, *MPL*, and *CALR* mutations in online edition).

Table 18-2 Somatic mutations seen in patients with ET, PV, and MF

Gene name	Mutation effect	PV (%)	ET (%)	MF (%)
<i>JAK2</i> (V617F)	JAK/STAT signaling	95–97	50–60	50–60
<i>JAK2</i> exon 12	JAK/STAT signaling	1–2	0	0
<i>CALR</i>	JAK/STAT signaling	0	25	30
<i>MPL</i>	JAK/STAT signaling	0	3–5	5–10
<i>CBL</i>	JAK/STAT signaling	Rare	Rare	5–10
<i>SH2B3/LNK</i>	JAK/STAT signaling	1–2	3–6	3–6
<i>ASXL1</i>	Epigenetic modification	2	2–5	10–35
<i>EZH2</i>	Epigenetic modification	1–2	1–2	7–10
<i>IDH1/2</i>	Epigenetic modification	1–2	1–2	5–6
<i>DNMT3A</i>	Epigenetic modification	5–10	1–5	8–12
<i>TET2</i>	Epigenetic modification	10–20	5	10–20
<i>SF3B1</i>	mRNA splicing	2	2	5
<i>SRSF2</i>	mRNA splicing	Rare	Rare	5–17
<i>U2AF1</i>	mRNA splicing	Rare	Rare	16
<i>ZRSR2</i>	mRNA splicing	Rare	Rare	1
<i>TP53</i>	DNA repair	Rare	Rare	Rare

The consequence is constitutive, ligand-independent activation of the JAK-STAT pathway and subsequent myeloid progenitor proliferation and differentiation, accounting for the phenotype of erythrocytosis, leukocytosis, and/or thrombocytosis often observed in MPNs. *JAK2*V617F is present in ~95% of PV patients and can be heterozygous, or homozygous in at least one-third of PV cases, due to acquired uniparental disomy of the region, including the mutated gene on chromosome 9p24.

Analysis of *JAK2* V617F-negative PV patients led to the identification of acquired-activating mutations in exon 12 of *JAK2*. Of note, unlike the more pleiotropic *JAK2*V617F allele, which is seen in a spectrum of myeloid malignancies, *JAK2* exon 12 mutations are found in *JAK2* V617F-negative PV, and most often are identified in patients with isolated erythrocytosis. *JAK2*V617F is identified in about 50% to 60% of ET and PMF cases. Patients

with post-ET/-PV MF have *JAK2* V617F as prevalent as the preceding MPN of ET/PV. Noncanonical, germ line *JAK2* variants have been identified in patients with “triple-negative” ET, as well as in families (*JAK2* V617I and *JAK2* R564Q) with hereditary thrombocytosis.

MPL

After discovery of *JAK2* V617F, much effort was expended in identifying other mutations important in diagnosis and pathogenesis in MPNs (Table 18-2). The next recurrent non-*JAK2* MPN mutations described were somatic activating mutations in the gene encoding the thrombopoietin receptor (*MPL*) in 3% to 5% of ET patients and 5% to 10% of those with PMF. These latter mutations have not been found in PV. *MPL* mutations (W515, S505) also lead to ligand-independent JAK-STAT activation and, predominantly, megakaryocyte proliferation. Noncanonical germ line and somatic *MPL* mutations have been identified in patients thought to have “triple-negative” ET. Germ line *MPL* (including S505, found to be either germline or somatic) mutations that lead to constitutive overexpression of the gene product have also been identified in several kindreds known to have hereditary thrombocytosis, a rare entity.

CALR

In late 2013, mutations in the calreticulin gene (*CALR*) were identified and are now known to be present in 25% to 30% of all ET and MF patients (Table 18-2). Mutations in *CALR* have been found infrequently in other myeloid neoplasms, including myelodysplastic syndrome (MDS), and MPN/MDS overlap syndromes. The mutations in *CALR* occur in the terminal exon 9 of the gene and result in a +1-base-pair frameshift in the reading frame. The two most common types of mutations include a 52-base frameshifting deletion (type 1/type 1-like) or a 5-base-pair insertion (type 2/type 2-like). Recent work by several laboratories suggests that the mutant *CALR* binds MPL, leading to activation of the JAK-STAT pathway. There are prognostic implications regarding the presence of the *CALR* mutation, as discussed in the ET and MF sections.

KIT D816V

KIT is the protein TK receptor for stem cell factor (SCF) and is expressed by mast cells, and accordingly, most cases of mastocytosis are associated with somatic-activating point mutations of *KIT*. The most common point mutations result from a Val for Asp substitution at codon 816 (D816V), which is found in ~90% of SM patients (skin, peripheral blood, and bone marrow) and results in ligand-independent activation of KIT, promoting mast cell proliferation and survival. A *KIT* juxtamembrane mutation

in codon 560 also has been described in a human mast cell line called HMC-1 and rarely is found in SM. Rare juxtamembrane and transmembrane variants of *KIT* point mutations also have been described, as well as alternative *KIT* D816 codon mutations such as D816Y/H/F/I.

CSF3R

In 2013, understanding of the genetic basis of CNL was improved with the demonstration of mutations in the gene encoding the receptor of colony-stimulating factor 3 (*CSF3R*) in a cohort of CNL patients. Maxson et al hypothesized that patients with CNL (and atypical CML) would harbor oncogenes that would be sensitive to kinase inhibition. Using a deep sequencing approach with coverage of 1,862 genes, they found that 16 of 27 (59%) harbored *CSF3R* mutations, including 8 of 9 with CNL. Two types of mutations were observed: membrane proximal mutations (point mutations in the extracellular or transmembrane region) and truncation mutations (frame-shift or nonsense mutations that truncate the cytoplasmic tail of *CSF3R*). The influence upon downstream signaling pathways, and, subsequently, sensitivity to kinase inhibition, differed depending on the type of mutation: truncation mutations activated the SRC family-TNK2 kinase signaling and showed sensitivity to dasatinib, whereas proximal mutations activated the JAK-STAT pathway. As proof of concept, a patient carrying a JAK-STAT-activating *CSF3R* mutation experienced clinical improvement in neutrophilic leukocytosis and thrombocytopenia when treated with ruxolitinib. In a murine model, transplantation with the most common mutation in CNL, *CSF3R* T618I, recapitulated a fatal MPN characterized by granulocytic proliferation and infiltration of the liver and spleen; JAK inhibition with ruxolitinib reduced the leukocyte count and spleen weight. Subsequently, in another study, 10 of 12 (83%) WHO-defined CNL cases were found to carry *CSF3R* mutations; 33% coexpressed *SETBP1* mutations.

Growth factor rearrangements: *PDGFRA*, *PDGFRB*, and *FGFR1*

The *PDGFRA* gene is located on the long arm of chromosome 4 (4q12) and has been implicated in the chronic eosinophilic syndromes as a result of a cryptic interstitial deletion at 4q12, leading to the juxtaposition and in-frame fusion of *FIP1L1* and *PDGFRA*. This deletion evades standard cytogenetic banding techniques, explaining why most cases of CEL apparently have a normal karyotype. Expression of *FIP1L1-PDGFR* transformed a murine hematopoietic cell line, was constitutively active in these cells, and led to increased STAT5 phosphorylation. Similar transforming properties were noted when *STRN-PDGFR*A or

ETV6-PDGFR4 fusion genes were transfected into murine hematopoietic cell lines. Several other partner genes have been implicated in the pathogenesis of *PDGFR4*-related neoplasms, including *BCR*, *ETV6*, *KIF5B*, and *CDK5RAP2*.

The *PDGFRB* gene is located on the long arm of chromosome 5 (5q31–33). In 1994, Golub et al were the first to characterize the t(5;12)(q31–q33;p13) translocation involving *ETV6* (12p13) and *PDGFRB* (5q33). Since then, more than 30 partner genes have been identified to collaborate in the development of *PDGFRB*-related neoplasms.

The molecular consequences of *FGFR1* rearrangements are remarkably well described for such an unusual disorder. In all *FGFR1*-related neoplasms, the N-terminal partner containing self-association motif is fused to the C-terminal Tyrosine kinase domain (TKD) of *FGFR1*. These fusion genes (*ZNF198FGFR1*), when expressed in primary murine hematopoietic cells, cause an MPN that recapitulates the human MPN phenotype. Furthermore, these constitutively active *FGFR1* fusion genes activate downstream effector molecules, such as PLC-g, STAT5, and PI3K/AKT.

Additional MPN mutations

A spectrum of somatic mutations in genes involved in various cellular processes has also been recurrently identified in MPNs (Table 18-2), including genes that regulate DNA methylation (*TET2*, *DNMT3A*, *IDH1/IDH2*), histone modification (*ASXL1*, *EZH2*), RNA splicing (*SF3B1*, *U2AF1*, *ZRSR2*, *SRSF2*), signal transduction (*LNK*, *CBL*, *NRAS*), and DNA repair (*TP53*). Identification of such mutations indicate clonality in those with “triple-negative” ET or MF and can be diagnostically useful. Prognostic implications of these mutations are discussed in the respective disease-associated chapters.

Additional contributions to disease pathogenesis

The presence of the *JAK2* V617F mutation across all subtypes of MPN, as well as *CALR* and *MPL* in both ET and MF, raises the question of what other factors contribute to the phenotypic heterogeneity within different MPNs that share the same mutation. Differences in allele burden, downstream intracellular signaling, host genetic background, age, sex, acquisition of other molecular mutations, including order, and the hematopoietic progenitor tissue type targeted by the mutation can all influence phenotype. A germ line haplotype (46/1, GGCC) at the 3' region of *JAK2* also has been associated with a three- to four-fold increased risk of developing a *JAK2* V617F mutant or *MPL* mutant MPN. *TERT* gene polymorphisms and other germ line predisposition loci affecting a multitude of cellular processes also contribute to an increased risk of MPN.

Overlapping features among the classical BCR-ABL1-negative MPNs

Mimicry has been a long-recognized feature of the MPNs, with regard to overlap in presentation, symptoms, physical exam findings, lab findings, and clinical consequences (Table 18-3). The constellation of MPN-related symptoms

Table 18-3 Laboratory, physical findings, and symptoms at presentation

Feature	PV	ET	MF (PMF/ post-ET/ PV MF)
Laboratory features			
Erythrocytosis	+++	Absent	Absent
Leukocytosis	Variable	Variable	Variable
Thrombocytosis	Variable	+++	Variable
Leukoerythroblastosis	Absent	Absent	+++
Decreased serum erythropoietin	+++	Variable	Absent
Elevated lactate dehydrogenase	Variable	Absent	Common
Hyperuricemia	Uncommon	Uncommon	Variable
Physical findings			
Splenomegaly	+	+	+++
Hepatomegaly	Absent	Absent	+
Plethora	++	Absent	Absent
Pallor	Absent	Absent	Variable
Disease-related symptoms (MPN-10)*			
Fatigue	84%	85%	94%
Early satiety	56%	60%	74%
Abdominal discomfort	48%	48%	65%
Inactivity	54%	60%	76%
Problems with concentration	58%	62%	68%
Night sweats	47%	52%	63%
Pruritus	46%	62%	52%
Bone pain	45%	48%	53%
Fever	17%	19%	24%
Weight loss	28%	33%	47%
Additional presenting features			
Erythromelalgia	+	++	Absent
Thrombosis	Variable	Variable	Variable
Hemorrhage	Variable	Variable	Variable
Portal hypertension	Variable	Variable	Variable

*See Geyer HL, Mesa RA, *Blood*. 2014;124:3529–3537.

+ to +++: Occasional to very common.

has been quantified and can be measured and tracked with validated MPN-specific, patient-reported outcome tools including MPN-10. In general, symptoms may be cytokine related, vascular in origin, and/or related to organomegaly. Close assessment of MPN symptom burden is recommended by clinical practice guidelines given impact on prognosis and therapeutic decision making. Physical exam findings may be absent. Organomegaly is variably present in ET and PV, and common in MF, along with other symptoms of signs of extramedullary hematopoiesis. Some degree of cytosis is variably present in each MPN. Vascular events are prevalent in ET, PV, and MF, peaking around the time of diagnosis and plateauing by the end of the first decade of the disease. Arterial events are more common than venous events, with exceptions observed in younger women, who can have predilection for unusual site thrombosis (hepatic/portal veins). Microvascular disturbances can impact quality of life and may reflect platelet hypersensitivity. Bleeding is less common than thrombosis, but present in each MPN, with multifactorial etiologies. Progression to MF in ET and PV occurs with longer disease duration; in all three MPNs, there is a risk for blast-phase transformation, typically via an MF phase.

Polycythemia vera

CLINICAL CASE



A 60-year-old male violinist presented with intractable pruritus. The patient's general practitioner noticed multiple skin excoriations but no rash. The patient was prescribed antihistamines and steroid cream. A week later, he returned, complaining of persistent pruritus, along with facial flushing and painful erythematous swelling of his fingers. Physical exam revealed erythematous swelling of both hands, multiple skin excoriations, and palpable splenomegaly. Vital signs including oxygen saturation were within normal limits. A complete blood count (CBC) showed the following: WBCs = $12 \times 10^9/L$, Hemoglobin (Hgb) = 17 g/dL, mean corpuscular hemoglobin: 85 fL, platelet count = $830 \times 10^9/L$. Additional blood tests showed a serum erythropoietin level of 2 U/L (normal, 7 to 20 U/L) and the presence of a JAK2 V617F mutation. The patient was phlebotomized and started on aspirin (81 mg by mouth once daily) with improvement in pruritus and also erythematous swelling of both hands.

Introduction

PV is the most common MPN in the United States, with an annual incidence rate of roughly 1.1 cases per 100,000

persons per year, a slight male predominance, and a median age at diagnosis in the seventh decade ($\sim 5\%$ of cases occur in those <40 years old). Radiation exposure and, rarely, environmental or toxic factors have been linked to the disease.

Diagnosis

Differential diagnosis

In adults with erythrocytosis, there is a broad differential diagnosis, including relative and secondary causes. (Table 18-4). An elevated hematocrit may result from either an increase in the total red cell mass (absolute) or a decrease in the total plasma volume (relative). The latter condition usually is due to moderate to severe intravascular dehydration, such as that due to diuretics, diarrhea, or loss of fluid into third spaces. In some cases (2.5% of healthy patients based on statistical distributions of laboratory ranges), an increased hematocrit may represent a normal variant.

A history of smoking or occupational exposure to hydrocarbon fumes may lead to arterial blood gas and carboxyhemoglobin determinations. Lung disease, cardiac disease, or sleep apnea should be considered. Physicians should ask about androgen replacement therapy or abuse. Inappropriate EPO production can occur in the setting of certain EPO-producing tumors (Table 18-4). Absolute erythrocytosis may occur in up to 15% of postrenal transplant patients; when treatment is required, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers are often effective. Some patients can have concurrent primary and secondary causes; presence of the JAK2 V617F allele supports a diagnosis of PV even in patients with concomitant secondary polycythemia.

Primary familial and congenital erythrocytosis is usually autosomal dominant and most commonly associated with low-serum EPO levels. Approximately 10% of such cases have been linked to germline truncating mutations of the EPO receptor that abrogate an important inhibitory domain and lead to constitutive EPO receptor signaling. In contrast, normal or high EPO levels are found in patients with Chuvash-type congenital polycythemia due to abnormalities in cellular oxygen sensing. This autosomal-recessive disorder was first recognized among the population of the Chuvash region of Russia and is associated with a high risk of thrombotic and hemorrhagic complications. Sporadic cases of Chuvash-type polycythemia with homozygous or compound heterozygous inheritance patterns subsequently have been identified among other ethnic groups. These patients have mutations involving a region of the *von Hippel-Lindau (VHL)* gene that

Table 18-4 Causes of secondary erythrocytosis

Congenital	
Mutant high oxygen-affinity hemoglobin	
Congenital low 2,3-bisphosphoglycerate	
Autonomous high-EPO production (including Chuvash-type polycythemia associated with <i>VHL</i> mutations)	
Autosomal dominant polycythemia (including truncating EPO receptor mutations)	
HIF2A (<i>EPAS1</i>) mutation	
Proline hydroxylase (<i>EGLN1</i>) mutation	
Congenital methemoglobinemia	
Acquired	
<i>Arterial hypoxemia</i>	
High altitude	
Cyanotic congenital heart disease	
Chronic lung disease	
Sleep apnea and hypoventilation syndromes	
<i>Other causes of impaired tissue oxygen delivery</i>	
Smoking	
Carbon monoxide poisoning	
Acquired methemoglobinemia	
<i>Renal lesions</i>	
Renal cysts	
Hydronephrosis	
Renal artery stenosis	
Renal transplantation	
<i>Miscellaneous tumors</i>	
Parotid tumors	
Cerebellar hemangiomas	
Hepatoma	
Renal cell carcinoma	
Uterine myomata	
Cutaneous leiomyomata	
Bronchial carcinoma	
Ovarian tumors	
Adrenal tumors	
Meningiomas	
Pheochromocytomas	
<i>Drugs and chemicals</i>	
Androgens	
ESAs (eg., epoetin alfa or darbepoetin alfa)	
Nickel, cobalt	

Modified from Pearson TC, Messiney M, *Pathol Biol (Paris)*. 2001;49:170-177.

is distinct from the autosomal-dominant *VHL* mutations associated with von Hippel-Lindau syndrome. The Chuvash-type *VHL* mutations impair the function of the *VHL* gene product to facilitate degradation of hypoxia-inducible factor 1 (HIF1), an oxygen-responsive transcriptional factor that upregulates EPO expression. More recent studies of families with autosomal-dominant heri-

table erythrocytosis have identified germ line mutations in the *HIF2A* gene that lead to defective oxygen sensing and resultant polycythemia; of note, these mutations are heterozygous and result in dysregulation of the HIF transcriptional complex. Another autosomal-dominant familial polycythemia is caused by germ line mutations in *proline hydroxylase domain 2 (PHD2)*. *PHD2* is an Fe(II)- and 2-oxoglutarate-dependent oxygenase that hydroxylates HIF2A to allow it to be targeted for ubiquitination and degradation by VHL. Finally, high-affinity hemoglobins may be found in those with a family history of erythrocytosis; such patients may be diagnosed by the identification of a low P50 value on hemoglobin-oxygen affinity curve testing.

Distinction between PV and secondary erythrocytosis is important both for prognosis and treatment because secondary polycythemia does not carry a risk of leukemic or fibrotic transformation and has a lower/unclear risk of thrombosis. Further, phlebotomy can be harmful when erythrocytosis is compensatory (in those with cyanotic congenital heart disease, chronic hypoxia, or high-affinity hemoglobins). Phlebotomy is only occasional necessary in secondary polycythemia, indicated to decrease blood viscosity and improve oxygenation when symptoms occur or prophylactically, especially when hematocrit values exceed 60%.

Diagnostic criteria for PV

The WHO revised diagnostic criteria for PV in 2016 (Table 18-5). Compared to the 2008 revision, the most significant change is in the lowering of the hemoglobin/hematocrit threshold. This change was made based on concerns that the prior Hgb/Hematocrit (Hct) 2008 criteria had lost sensitivity in striving for extreme specificity. Prior studies supporting this change suggested that cohorts of *JAK2*-positive ET were reclassified as having

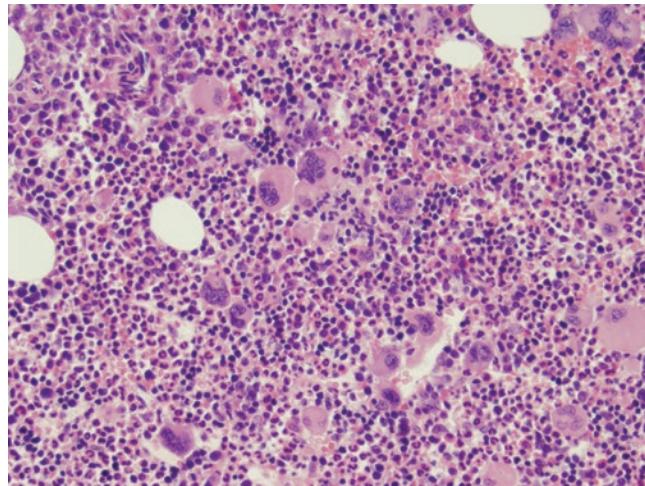
Table 18-5 WHO 2016 PV diagnostic criteria

Major	<ol style="list-style-type: none"> Hemoglobin >16.5 g/dL in men (or Hct > 49%), >16.0 g/dL in women (or Hct > 48%), or >25% increase in red cell mass Bone marrow biopsy with characteristic features (panmyelosis, pleomorphic megakaryocytes) Presence of <i>JAK2V617F</i> or <i>JAK2</i> exon 12 mutation
Minor	Serum erythropoietin level below the reference range for normal
Diagnosis	All three major criteria or first two major criteria and the minor criterion

Adapted from Arber DA et al, *Blood*. 2016;127(20):2391-2405.

PV when additional testing, such as red cell mass studies or bone marrow biopsies, were performed. Another study of those with “masked PV” (*JAK2* mutations and characteristic bone marrow features but Hgb values below the 2008 threshold) distinguished this group from those with ET if the Hgb values were ≥ 16 g/dL or 16.5 g/dL in women and men, respectively. The identification of *JAK2* V617F or *JAK2* exon 12 mutations remain a major criterion (Table 18-5). A bone marrow is now required as a major criterion for diagnosis. Characteristic bone marrow features include panmyelosis, an increase in pleiomorphic megakaryocytes (ranging from small to medium to large sizes), and absent iron stores (Figure 18-1). The bone marrow can be deferred per WHO if prior WHO 2008 Hgb/Hct thresholds are observed (Hgb > 16.5 g/dL and 18.5 g/dL in women and men, respectively), in the setting of other major criteria. The bone marrow can be prognostic, as up to 20% may have grade 1 fibrosis at diagnosis, which predicts for a higher rate of overt fibrotic progression. Additionally, at diagnosis, ~15% of karyotypes from PV patients contain nonrandom chromosomal abnormalities, which may be prognostically important. The only remaining minor criterion is a subnormal EPO level, observed in ~85% of patients. Serum EPO levels within the normal range also occur in PV, especially when EPO levels are not measured until after the patient has undergone initial therapeutic phlebotomy.

Figure 18-1 PV bone marrow biopsy. The core shows a hypercellular marrow for age with panmyelosis (proliferation of the erythroid, granulocytic, and megakaryocytic lineages). Megakaryocytes are increased and include frequent hyperlobated forms. Source: ASH Image Bank/Elizabeth L. Courville.



Disease course and prognosis

In the short term, patients with PV have a risk of thrombotic events, either microvascular or macrovascular, as well as risk of hemorrhagic events. Additionally, the symptomatic burden may be bothersome. Progression to fibrosis or leukemia is possible, typically later in the disease course.

Vascular events in PV

Vascular events are a major cause of morbidity and death. Data from the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) study revealed a thrombotic complication rate of 5.5 events per 100 patients per year at a median follow-up time of 2.7 years. Two-thirds of those events were arterial, and one-third were venous. The risk of a thrombotic complication in the ECLAP cohort was increased in PV patients > 65 years old (hazard ratio [HR], 8.6), with a history of prior thrombosis (HR, 4.85), or > 65 years old and with thrombosis (HR, 17.3); accordingly, age and thrombosis history represent the major factors used to assess thrombotic risk in PV patients. In addition, cardiovascular morbidity and mortality in PV were linked significantly to smoking, diabetes, and congestive heart failure. Subsequent multicenter retrospective studies as well as a prospective randomized study reported overall event rates near 2.6% to 2.7% per year, possibly reflecting earlier diagnosis and more aggressive treatment approaches. In a population-based cohort study, the highest rate-ratios for thrombosis, in all groups, were observed shortly after diagnosis, but persisted through follow-up.

Thrombosis risk in PV has a multifactorial set of etiologies. Beyond age and prior thrombosis history, there is a contribution from uncontrolled erythrocytosis, because lowering hematocrit has shown to be protective. In addition, there are contributions from cardiovascular risk factors, including hypertension, and differences in risk according to sex (venous events more likely in women). Platelet and granulocyte activation (as well as leukocytosis) likely contribute to the pathogenesis as well. Thrombocytosis itself does not associate with thrombosis risk but, rather, bleeding risk. Increased *JAK2* allelic burden may also contribute. In keeping with contributions from acquired somatic mutations, clonal hematopoiesis itself associates with an increase in cardiovascular risk, mediated through inflammatory stress. A number of other potential biomarkers have been reported but are not used routinely in clinical practice.

Bleeding rates are lower than thrombosis rates and also with multifactorial contributions. In some cases, acquired von Willebrand's disease may be present. Thrombocytosis correlates more so with bleeding than thrombosis. Anti-

platelet and anticoagulant use can increase risk, and bleeding event rates are near 8% following surgery.

Post-PV MF

Patients with PV can progress to post-polycythemic MF (post-PV MF) or to overt acute myeloid leukemia (AML) (ie, MPN-blast phase [BP]) (Table 18-6). Post-PV MF typically develops in individuals with longstanding disease duration (>10 years). Changing features in this setting can include loss of a phlebotomy requirement, anemia, or cytopenias in the setting of cytoreduction, increasing leukocytosis, progressive splenomegaly, a change in symptoms (including weight loss, night sweats, bone pain, fever), and, finally, increasing marrow fibrosis. Risk factors for MF progression include advanced age at diagnosis, disease duration, leukocytosis ($\geq 15 \times 10^9/L$) at diagnosis, baseline bone marrow fibrosis, an increased *JAK2* allelic burden (>50%), and, possibly, additional somatic mutations, including *ASXL1*, *IDH*, and *SRSF2*.

MPN-blast phase

PV can transform to MPN-BP, often through an MF phase, or rarely via a PV phase. This transformation is suspected when there are >20% blasts in the marrow or blood (Table 18-6). A baseline rate of MPN-BP from PV is modest, as MPN-BP occurs in roughly 1% to 3% of patients treated with phlebotomy alone. In contrast, cer-

tain therapies, including Phosphorus-32 (^{32}P) treatment, chlorambucil, possibly busulfan, and alkylating agent combinations have been associated with increased risk of transformation to MPN-BP (up to 15-fold increased risk in randomized Polycythemia Vera Study Group [PVSG] trials). The ECLAP study noted a higher rate of AML/MDS transformation with pipobroman use; this agent is no longer available in the United States but is still available for use in Europe and elsewhere. Although early observational studies suggested that MPN-BP might be increased in patients receiving hydroxyurea (HU), the largest prospective PV study to date, the ECLAP study, enrolled 1,638 patients and noted no increase in MPN-BP in patients treated with HU, with a median follow-up time of 8.4 years after PV diagnosis and 2.5 years after study enrollment. Neither interferon alpha (IFN) α nor anagrelide is leukemogenic. Finally, acquired somatic mutations may increase risk, including *ASXL1*, *IDH*, and *SRSF2*.

Prognosis

PV is a chronic disease that is incurable without stem cell transplant, though transplant is almost never done in the PV phase. In the contemporary era, age at diagnosis, leukocyte count at diagnosis, and thrombosis history influence prognosis, with a life expectancy between ~11 and 28 years depending on risk grouping (Table 18-7). As with MF

Table 18-6 Criteria of progression of PV and ET

Type of progression	Criteria	Details
Post-ET or post-PV myelofibrosis (both major criteria and two minor criteria required)	Major criteria	Documentation of a previous WHO diagnosis of ET or PV
		Bone marrow fibrosis grade 2 to 3 (on 0 to 3 scale) or grade 3 to 4 (on 0 to 4 scale)
	Minor criteria	PV: Anemia or sustained loss of requirement of either phlebotomy (in the absence of cytoreductive therapy) or cytoreductive treatment for erythrocytosis (PV)
		ET: Anemia and a 2 g/dL decrease from baseline Hgb
		A leukoerythroblastic peripheral blood picture
		ET: Increased LDH above reference range
		Increasing splenomegaly defined as either an increase in palpable splenomegaly by 5 cm (from the left costal margin) or the appearance of newly palpable splenomegaly
		Development of one of three constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fevers $>37.5^\circ\text{C}$
MPN-BP (either criterion)	Bone marrow	$\geq 20\%$ blasts
	Peripheral blood	$\geq 20\%$ blasts that last for at least 2 weeks

and AML, additional somatic mutations in PV may further influence risk (*ASXL1*, *IDH*, and *SRSF2*).

Management

The goals of therapy in PV include providing symptom relief, reducing risk for incident/recurrent thrombosis (and hemorrhage), and, ideally, preventing or delaying transformation. The latter goal is difficult to achieve with current therapies. Management has been historically guided by vascular risk, but it is important to incorporate symptom burden into treatment decisions (Figure 18-2).

Hematocrit control

Phlebotomy is a mainstay of treatment. The target goal for phlebotomy has been evaluated by the CYTO-PV study. Patients were randomized between a target hematocrit of 45% to 50% or less than 45% and were allowed to use cytoreductive therapy. In those with a hematocrit target below 45%, there was a nearly 4-fold reduction in risk of cardiovascular (CV) death and major thrombosis. This study had a significant impact on practice, and a hematocrit of 45% or less is the target hematocrit. While it is not data driven, many use a hematocrit of 42% or less as a target hematocrit in women. Phlebotomy to the point of iron deficiency may be associated with reactive thrombocytosis,

Table 18-7 PV survival, based on risk factors

Age, years	>67 (5 points)
	57–66 (2 points)
	<57 (0 points)
Leukocytes	>15 × 10 ⁹ /L (1 point) vs <15 × 10 ⁹ /L
Prior thrombosis	Yes (1 point) vs no (0 points)
Risk group point cutoffs/ survival	Sum above points. Median survival: Low risk (0 points): 27.8 years Intermediate risk (1 to 2 points): 18.9 years High risk (≥3 points): 10.9 years

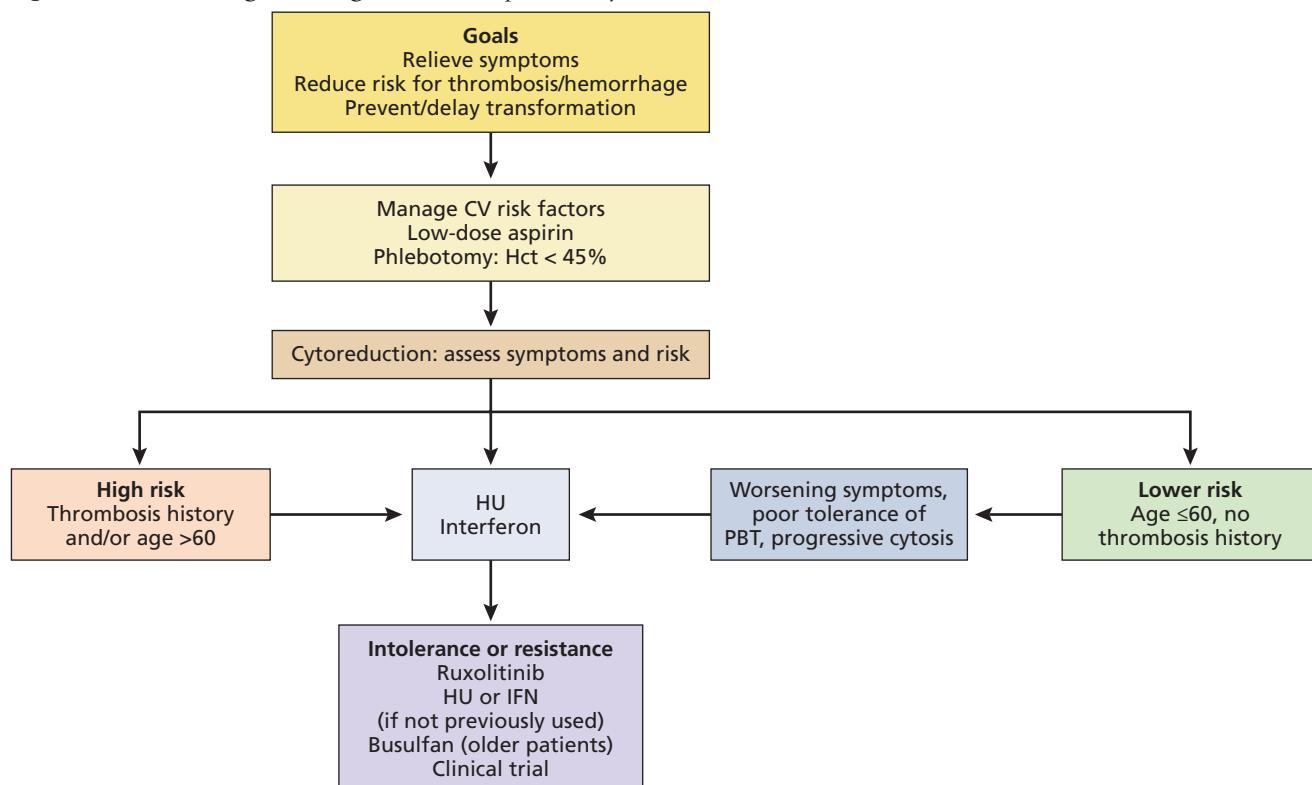
Adapted from Tefferi A et al, *Leukemia*. 2013;27:1874–1881.

although the thrombohemorrhagic risk in this clinical setting has not been delineated. Iron supplementation should be avoided to prevent undue elevation in hematocrit levels.

Antiplatelet therapy

The ECLAP study, a double-blind randomized trial, compared low-dose aspirin with placebo among 518 patients who had no indication for anticoagulation and no pre-existing clear indication or contraindication to aspirin

Figure 18-2 PV management algorithm. PBT, phlebotomy.



therapy. The study demonstrated that low-dose aspirin (ie, 100 mg/d) reduces the rate of thrombosis and cardiovascular deaths in those receiving standard phlebotomy and supportive care. The aspirin-treated group experienced 60% fewer major thromboses and cardiovascular deaths (3.2% vs 7.9% absolute incidence) after roughly 3 years of follow-up. The ECLAP trial observed only a modest increase in epistaxis and no increase in major bleeding on low-dose aspirin. Low-dose aspirin also can effectively control erythromelalgia and other vasomotor symptoms in most patients. Prior PVSG trials showed that higher doses of aspirin (ie, 500 to 900 mg/d) offer no added benefit but increased the risk of bleeding complications, especially when combined with dipyridamole. The role of clopidogrel and other anti-platelets/dosing regimens is not well defined.

Cytoreduction

Cytoreduction is typically reserved for high-vascular-risk patients (aged ≥ 60 years and/or with a thrombosis history). Additional indications include poor tolerance of phlebotomy, symptomatic thrombocytosis, progressive leukocytosis, symptomatic splenomegaly, and uncontrolled symptoms impacting quality of life.

Hydroxyurea

HU, a ribonucleotide reductase inhibitor, is most commonly used for those requiring cytoreductive therapy. HU emerged as the cytoreductive of choice based on historical PVSG studies showing a lower rate of thrombosis compared to phlebotomy alone and lower risks of secondary leukemia compared to chlorambucil and ^{32}P . The mutagenic and leukemogenic potential of HU has been a subject of concern; but overall, the AML/MDS risk with chronic HU therapy appears lower in magnitude than with other cytoreductive agents, such as chlorambucil, ^{32}P , pipobroman, and busulfan. Nevertheless, because of uncertainty regarding these concerns, HU often is avoided in younger adults, and it should be used only after a thorough discussion of the potential risks and benefits. Additional adverse effects of HU include cytopenias, gastrointestinal disturbances, and, less commonly, chronic mucocutaneous ulcers. The prevalence of HU intolerance (hematological or nonhematological) or resistance (uncontrolled Hct, leukocytes, platelets, or spleen size) despite a sufficient dose/duration is $\sim 10\%$ to 20%. Whether or not an active phlebotomy requirement despite HU treatment increases thrombosis risk remains to be seen. Among the factors defining intolerance, the development of cytopenias on HU (intolerance) may have the most negative influence on prognosis. Intolerance or resistance is an indication to move on to second-line therapy.

Interferons

Clinical practice guidelines recommend interferons as one potential frontline option in those who require cytoreduction (especially younger patients). Both short-acting and longer-acting IFNs have consistently demonstrated ability to control erythrocytosis, leukocytosis, and/or thrombocytosis. Adverse events, including fatigue, mood change, myalgias/flu-like symptoms, optic changes, emergence of autoimmunity, and neuropathy, have tempered enthusiasm. Recent studies with pegylated IFN α have demonstrated significant clinical efficacy, including clinical and molecular remissions in a substantial proportion of patients with improved tolerability, though adverse events (AE) are still observed. Current trials are aimed at assessing the efficacy and safety of pegylated IFN α in a larger cohort of PV patients, either when compared directly to HU, or when used as salvage following HU. Two large, global phase 3 clinical trial programs of IFN use in patients with PV are ongoing. The first is a randomized trial of newly diagnosed, high-risk patients with PV, randomizing between pegylated IFN α 2a and HU. A companion phase 2 study has completed enrollment in high-risk patients after experiencing HU resistance/intolerance. In parallel, there is a randomized phase 3 for newly diagnosed, high-risk PV patients with a monopegylated IFN proline, ropeginterferon. At the time of this writing, compared to HU, these agents appear noninferior. IFN α therapy is safe during pregnancy, in contrast to HU, which may be teratogenic (although experience from sickle cell anemia populations suggests that HU is a low-risk agent, so abortion is not justified solely based on inadvertent fetal HU exposure).

JAK inhibition

Ruxolitinib was approved for second-line use in the setting of an inadequate response to HU. The RESPONSE trial evaluated ruxolitinib vs best alternative therapy (BAT) in a cohort of PV patients with HU intolerance/resistance, active phlebotomy needs, and splenomegaly. Ruxolitinib-treated patients were more likely to meet the primary endpoint comprised of spleen volume reduction and hematocrit control, compared to those treated with BAT. There was also greater improvement in PV-related symptoms and a trend for a reduced number of thrombotic events in the ruxolitinib arm, though this was not a primary endpoint, nor was the study powered to detect differences between treatment groups. Longer-term follow-up shows durability in primary responders. A second phase 3 study, in similar patients, but lacking splenomegaly, also showed superior hematocrit control in ruxolitinib-treated patients, compared to BAT (RESPONSE-2). Myelosuppression is less common in PV compared to MF, but

AEs include weight gain, increase in cholesterol, increase in skin cancer in at-risk patients, and increase in infections such as zoster. Ruxolitinib may be particularly effective for control of pruritus.

Additional therapeutic considerations

Thrombotic events are managed with therapeutic anticoagulation in a similar manner to other patients who present with acute thrombosis. Blood count control, including phlebotomy to normalize the hematocrit, should be initiated if patients have a hematocrit >45%. The utility of platelet-pheresis for thrombocythemic patients with acute thrombosis and the optimal target platelet count after depletion is unknown. Antiplatelet therapy in addition to warfarin may be useful in selected cases of PV-associated arterial thrombosis, but only after the acute event is stabilized with full anticoagulation and only if the potential additive risk of bleeding is considered acceptable. Duration of anticoagulation continues to be unclear.

Abdominal vein thrombosis as well as Budd-Chiari syndrome, portal vein occlusion, and mesenteric vein thrombosis are all more frequently encountered in patients with MPNs. Of the MPN subtypes, these events may be most commonly observed in PV. In some cases, an MPN is entirely latent. The natural history of individuals not meeting WHO diagnostic criteria for MPNs but having an abdominal vein thrombosis and driver mutation remains unclear. In general, indefinite anticoagulation is recommended, and if cytosis is present, cytoreduction is indicated. Because these patients may have complications from portal hypertension (HTN), comanagement with hepatology is often needed, especially in those with esophageal varices.

The PV symptom burden can be considerable, even in traditionally lower-risk patients (Table 18–6). Therefore, low-risk, but symptomatic, patients may require therapy beyond phlebotomy and aspirin. Problematic symptoms can include fatigue, pruritus, and symptoms from splenomegaly. Pruritus may be a particularly disturbing symptom that often is unresponsive to phlebotomy or cytoreductive therapy. JAK inhibition with ruxolitinib has been helpful for some patients; additionally, antihistamines, psoralen and ultraviolet A phototherapy, cholestyramine, or selective serotonin reuptake inhibitors (ie, paroxetine) may provide symptomatic relief.

Cytoreductive therapy with HU or IFN ($\text{IFN}\alpha$ or pegylated $\text{IFN}\alpha$) may help in refractory cases. Painful splenomegaly and unacceptable hypercatabolic symptoms usually require treatment with HU or IFN ($\text{IFN}\alpha$ or pegylated $\text{IFN}\alpha$).

PV (as well as ET or MF) patients undergoing elective surgical procedures may have an increased risk of bleed-

ing and/or thrombosis, even despite blood count control and prophylaxis. Emergency surgical procedures should proceed as necessary, but with awareness of a higher risk of vascular complications, particularly in those with uncontrolled thrombocytosis, erythrocytosis, or leukocytosis. Preparation for elective procedures includes hematocrit control for those with PV and, quite possibly, cytoreduction to control leukocytosis/thrombocytosis, depending on the nature of the procedure. Hematologists should discuss whether or not antiplatelets should be held prior to the surgery. Provided no contraindications, ideally, patients are managed with VTE prophylaxis after surgery. Hematologists and the surgeon can decide upon the timing of re-initiation of antiplatelet therapy.

KEY POINTS

- PV is a clonal disorder associated with *JAK2 V617F* or exon 12 mutations.
- PV must be differentiated from relative/secondary causes of erythrocytosis.
- PV patients may exhibit a range of symptoms that can be cytokine related, vascular in origin, or due to progression.
- Thrombosis is the major cause of morbidity and mortality in the first decade of the disease, while progression to post-PV MF or BP becomes a concern in the second decade.
- All patients should have CV risk factor modification, low-dose aspirin, and phlebotomy for a Hct target of 45% or less.
- Cytoreductive therapy (HU or pegylated IFN) for symptomatic and higher-risk PV patients should be considered.
- Ruxolitinib is approved for PV patients with inadequate (resistant or intolerant) response to HU.

Essential thrombocythemia

CLINICAL CASE

A 40-year-old, previously healthy landscaper complained of increased fatigue and migraines. A CBC revealed a platelet count of $1,062 \times 10^9/\text{L}$. She was then referred to a hematologist, whose evaluation included iron studies and inflammatory markers that were within normal limits. Fluorescence in situ hybridization (FISH) for *BCR-ABL1* and *JAK2 V617F* testing were negative, but she did have a calreticulin (*CALR*) mutation. Her bone marrow was slightly hypercellular with mature megakaryocytic hyperplasia and no reticulin fibrosis.

Introduction

ET is about as prevalent as PV, with approximately 150,000 cases in the United States (estimated from claims databases) and an annual incidence rate of approximately 0.5 to 1.5 cases per 100,000 persons per year. The median age at diagnosis is approximately in the mid-seventh decade; however, the distribution of cases is quite broad and also includes patients diagnosed in the third or fourth decade. There is a predominance of women with ET compared to men. Morbidity and mortality from ET in the first decade of disease are predominantly related to thrombotic, vasomotor, and, less commonly, hemorrhagic complications. Longstanding ET, similar to PV, can progress to either post-ETMF or blast phase.

Diagnosis

Secondary causes are more common than primary causes of thrombocytosis and include reactive states due to iron deficiency, infection, inflammation, surgery (especially the postsplenectomy state), trauma, tissue injury or infarction, and malignancy. The absolute value of the platelet count usually does not distinguish reactive thrombocytosis from ET, although reactive conditions infrequently cause elevations in platelet counts of $>2,000 \times 10^9/L$. It is also important to exclude other MPNs, which can all present with thrombocytosis (especially “occult” PV, PMF, and CML) and MDS [especially 5q- syndrome, chromosome 3(q21;q26) abnormalities, or MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T, formerly RARS-T)]. Hereditary/familial thrombocytosis is also in the differential diagnosis; as noted, germline *MPL* and *JAK2* mutations have been described in hereditary thrombocytosis and in those initially suspected to have “triple-negative” ET.

Diagnostic criteria for ET

The WHO revised the diagnostic criteria for ET in 2016 (Table 18-8). The most important difference is the inclusion of *CALR* mutations as representative clonal markers. As discussed in the MF section, it is important to distinguish from prefibrotic/early MF, given prognostic implications.

Blood and bone marrow findings

Anemia and leukocytosis are less common in ET, and the presence of these, along with increased Lactate dehydrogenase (LDH), should raise suspicion for prefibrotic/early MF. The peripheral blood smear often is notable for large or giant platelets with occasional eosinophils, basophils, or circulating megakaryocyte fragments. Marrow evaluation is important in suspected ET cases to assess for character-

Table 18-8 WHO 2016 Diagnostic criteria for ET

Major	1. Sustained platelet count $\geq 450 \times 10^9/L$
	2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with mature megakaryocytes, with hyperlobulated nuclei; no significant increase or left shift of neutrophil granulopoiesis or erythropoiesis and very rarely grade 1 increase in reticulin
	3. Not meeting WHO criteria for PV, PMF, CML, MDS, or another myeloid neoplasm
	4. Presence of <i>JAK2V617F</i> , <i>CALR</i> , or <i>MPL</i> mutation
Minor	Presence of a clonal marker or exclusion of reactive thrombocytosis
Diagnosis	All four major criteria, or the first three major criteria and minor criteria

Adapted from Arber DA et al, *Blood*. 2016;127(20):2391–2405.

istic histopathologic features. Increased numbers and clusters of large megakaryocytes with hyperploid nuclei are seen in most marrow samples, and the bone marrow may be normocellular or only mildly hypercellular (Figure 18-3). Megakaryocyte atypia and hypercellularity/left-shifted granulopoiesis should not be seen. Significant reticulin and collagen fibrosis are minimal or absent. If $>15\%$ ring sideroblasts are present, MDS/MPN-RS-T rather than ET must be considered.

Disease course and prognosis

The disease course of ET can range from a near-normal life expectancy to significant morbidity and mortality and death

Figure 18-3 ET bone marrow. The marrow is hypercellular for age with increased megakaryocytes. The megakaryocytes are dispersed throughout the marrow and include frequent large forms with abundant cytoplasm and deeply lobated nuclei. Source: ASH Image Bank/Elizabeth L. Courville.

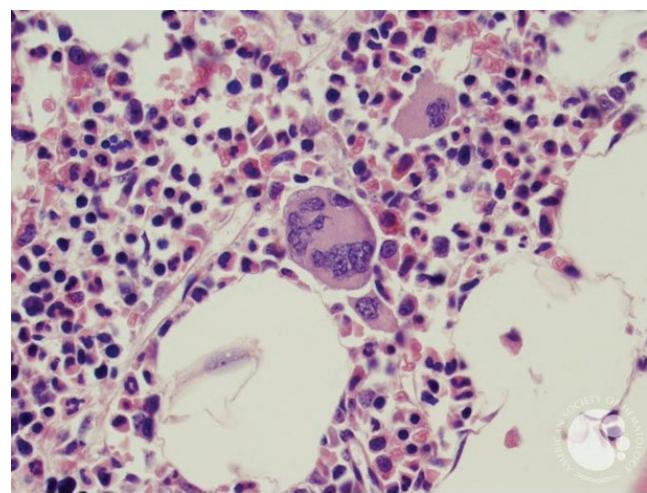


Table 18-9 Current ET vascular risk classification

Category	Age (years)	Thrombosis history	JAK2 status	Yearly thrombosis risk
Very low risk	≤60	None	Negative	~0.4% to 0.6%
Low risk	≤60	None	Positive	~0.8% to 1.6%
Intermediate risk	>60	None	Negative	~1.4% to 1.6%
High risk*	>60	Positive	Positive	~2.5% to 4%

*High-risk patients are either older than 60 with JAK2 mutations, or any patient with a prior thrombotic event.

Adapted from Gerdts AT, Mesa R, *The Hematologist*. 2017;14(6) (<http://www.hematology.org/Thehematologist/Features/7895.aspx>).

from vascular events or progressive disease. A common misperception is that ET patients are asymptomatic—recent studies demonstrate that ET patients, although generally less symptomatic than PV or MF, can have significant vascular and cytokine-related symptoms that impact quality of life.

Vascular events in ET

Up to 30% of ET patients have had a thrombotic event prior to or around the time of an official diagnosis. In a population-based study, the hazard ratio for overall thrombosis was 3.5, 2.2, and 1.7 at 3 months, 1 year, and 5 years from diagnosis, compared to controls. As in PV, older age and prior thrombosis history influence risk. The driver mutational profile is also important, because lower rates of thrombosis have been observed in patients with *CALR* mutations, compared to those with *JAK2* mutations. Current risk classification is therefore based on age, thrombosis history, and *JAK2* mutational status (Table 18-9). Regardless of formal risk classification, it is imperative to manage cardiovascular risk factors, including smoking, hypertension, diabetes, and dyslipidemia. As in PV, leukocytosis is likely to increase thrombosis risk. Current data does not suggest that the absolute platelet number predicts thrombosis risk; rather, studies have found a correlation between bleeding risk and extreme thrombocytosis, especially when $>1,500 \times 10^9/L$. In some cases, this risk is due to development of acquired von Willebrand disease.

Disease progression post-ET myelofibrosis

As with PV, progression to MF is suspected in the presence of changing symptoms, new or progressing splenomegaly, development of anemia, and increased LDH, along with blood smear and bone marrow changes and progression of fibrosis (Table 18-6). The risk of progression in WHO-defined ET nears 10% at 15 years. In those with a more rapid progression, it is possible that the original diagnosis was early/prefibrotic MF. Apart from disease duration, leukocytosis, anemia, and advanced age may influence MF progression rates. With regard to mutational status, it is possible that MF progression rates are increased in those with *CALR* mutations. Further, when identified in ET, *SH2B3*,

SF3B1, *U2AF1*, *TP53*, *IDH2*, or *EZH2* are considered adverse variants that may impact MF progression.

MPN-blast phase

Diagnostic criteria for MPN-BP from ET are also shown in Table 18-6. In the absence of leukemogenic therapy (radiophosphorus, alkylators), progression to MPN-BP directly from ET, without an intervening post-ET MF phase, is uncommon. The risk of MPN-BP from ET in WHO-ET was approximately 2% at 15 years. As above, adverse variants identified in ET (*SH2B3*, *SF3B1*, *U2AF1*, *TP53*, *IDH2*, or *EZH2*) may impact risk for leukemia.

Survival

In a study of 800 WHO-defined ET patients, multivariate analysis found age, leukocytosis, and prior vascular events as most prognostic for survival (Table 18-10). Another large study suggested a median life expectancy of near 20 years, though inferior to age/sex-matched controls. Driver mutational status did not impact survival in this study. In keeping with potential influence on MPN-BP and post-ETMF transformation, adverse variants impacted survival compared to those without such mutations.

Management

Goals of therapy in ET also include providing symptom relief, reducing risk for incident/recurrent thrombosis (and

Table 18-10 ET survival in WHO-defined ET (three groups)

Parameter	0 points	1 point	2 points
Age, years	<60	—	≥60
Leukocytes ($\times 10^9/L$)	<11	≥11	—
History of thrombosis	No	Yes	—
Low risk	0 points:	Median survival not reached (>30 years)	
	1 to 2 points:	Median survival: 24.5 years	
	3 to 4 points:	Median survival: 13.8 years	

Adapted from Passamonti F et al, *Blood*. 2012;120(6):1197–1201.

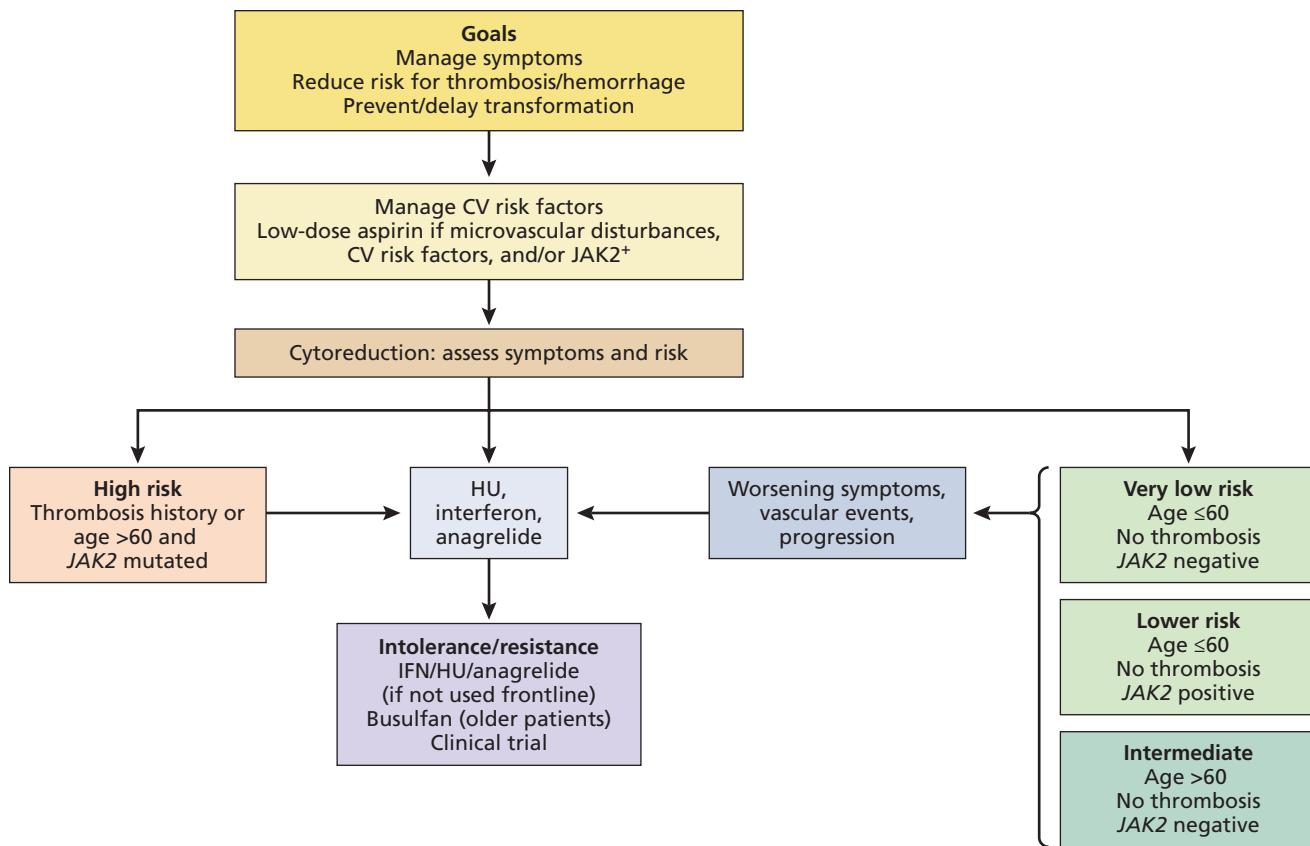


Figure 18-4 ET management algorithm.

hemorrhage), and preventing transformation. As with PV, the latter goal is not yet achievable with current medical therapy. Although management is strongly influenced by vascular risk assessment, it is important to also incorporate symptom assessment into therapeutic planning. (Figure 18-4).

Antiplatelet therapy

Unlike in PV, there are no randomized studies supporting use of aspirin in ET patients. A meta-analysis suggested inconsistent evidence and an uncertain benefit-risk ratio. Rather, aspirin use in ET is selective and certainly considered in the presence of CV risk factors. Additionally, vasomotor symptoms and erythromelalgia are often responsive to therapy with aspirin. Mutational status may influence decision making, because a retrospective study suggested that patients with *CALR*-ET did not have reduction in thrombosis risk, but rather, possibly, an increase in bleeding risk. In those with extreme thrombocytosis, excluding acquired von Willebrand disease is important prior to recommending aspirin. As for PV, the role of clopidogrel or other antiplatelet agents in ET is unknown. Whether aspirin is necessary in asymptomatic

low-risk patients with ET is unknown and remains a point of clinical judgment.

Cytoreduction

Cytoreduction is considered in those that are high risk by revised-IPSET or have problematic ET-related symptoms. Options for cytoreduction include HU, interferons, and anagrelide.

Hydroxyurea

The first randomized trial of 114 patients of HU versus placebo demonstrated a decrease in thromboembolic events in high-risk ET patients treated with HU. A follow-up report of this study (median treatment time, 73 months) revealed a continued benefit for HU: 45% of patients in the control group suffered a thrombotic event versus 9% of patients in the HU group. Of note, 1.7% of control patients and 3.9% of the group receiving HU developed secondary myeloid malignancies (AML/MDS), a difference that was not statistically significant.

A second important randomized study (PT-1 trial) included 809 high-risk ET patients treated with aspirin and

randomized to either HU or anagrelide, with a goal platelet count of $<400 \times 10^9/L$. After a median follow-up of 39 months, despite similar platelet count control, compared with HU plus aspirin, patients receiving anagrelide plus aspirin had increased rates of arterial thrombosis (but a lower rate of venous thrombosis), serious hemorrhage, and development of marrow fibrosis. Patients receiving anagrelide were more likely to withdraw from their assigned treatment because of toxicity or treatment failure. Taken together, these studies supported HU as a front-line cytoreductive in high-risk patients.

Anagrelide

A subsequent study compared anagrelide to HU in those with WHO-defined ET (ANAHYDRET); this noninferiority study (excluding aspirin in the anagrelide arm with concerns of intensifying the antiplatelet properties of anagrelide) demonstrated no significant difference between HU and anagrelide with regard to rate of major/minor thrombosis, hemorrhage, or discontinuation rates. It has been suggested that use of WHO-ET criteria, rather than PVSG criteria (which may have included patients with leukocytosis, a risk factor for thrombosis), inclusion of newly diagnosed/untreated patients, and restriction of aspirin use may have accounted for differences in study outcomes when comparing ANAHYDRET to PT-1. Practice patterns and guidelines vary on which agent to use first, with anagrelide as a potential front-line therapy per National Comprehensive Cancer Network (NCCN) and second-line therapy per ELN.

Interferons

Both short-acting and longer-acting IFNs have demonstrated efficacy in ET and are considered as front-line options. Recent studies with pegylated IFN α have demonstrated significant clinical efficacy, including hematological and molecular responses (in both *JAK2* and *CALR*-mutant ET) in a substantial proportion of patients. These experiences have been reported in previously treated ET patients in clinical trial and real-world settings. Current trials are aimed at assessing the efficacy and safety of pegylated IFN α in newly diagnosed, high-risk ET, compared to HU. A second phase 2 study evaluated salvage use of pegylated-interferon in those with prior HU resistance or intolerance. IFN α therapy is considered reasonably safe during pregnancy, in contrast to HU and anagrelide, which may be teratogenic.

JAK inhibition

Ruxolitinib was active in a nonrandomized phase 2 study of those with HU failure and decreased thrombocytosis,

ET-related symptoms, and splenomegaly. However, a subsequent randomized phase 2 study (MAJIC-ET), comparing ruxolitinib to best therapy in those with HU resistance/intolerance, did not demonstrate any significant differences in complete response, thrombosis, hemorrhage, or transformation rates. Some symptoms, such as pruritus, were improved in those treated with ruxolitinib. Anemia and infections were more common in those treated with ruxolitinib. Based on this study, ruxolitinib is not yet recommended for use in ET.

Additional therapeutic considerations

As in PV, acute management of vascular events is heterogeneous. Historically, in the setting of acute arterial or venous events, emergency plateletpheresis was a consideration to reduce the platelet count if extremely high, but this is not a data-driven practice. Anticoagulation is indicated for those with venous events, but the type of anticoagulant and duration is still unclear. Indefinite anticoagulation is typically reserved for patients with abdominal vein thrombosis or recurrent thromboses. In either circumstance, the patient should be monitored closely for bleeding while receiving anticoagulation. As in PV, while management is guided by vascular risk, lower-risk patients with uncontrolled symptoms may be candidates for cytoreduction.

Pregnancy

MPNs may increase the risk of miscarriage, abruptio placentae, preeclampsia, and intrauterine growth retardation, as well as maternal VTE and/or hemorrhage. Based on the age distribution of MPN patients, the pregnancy literature primarily includes women with ET, as compared to PV and MF. Consensus guidelines advise on management strategies, though none are proven to improve outcomes. In low-risk pregnancies, it is recommended to control the hematocrit in patients with preexisting PV to $<45\%$ or a mid-gestation-specific range, whichever is lower. Aspirin is recommended during the antepartum, and prophylactic low-dose molecular-weight heparin may be recommended in the postpartum period. High-risk pregnancies are defined by prior thrombosis or hemorrhage attributed to MPN, previous pregnancy complications, or extreme thrombocytosis ($>1,500 \times 10^9/L$). High-risk patients may require low-molecular-weight heparin throughout pregnancy, while monitoring for bleeding complications. If there has been previous major bleeding, avoidance of aspirin may be necessary. If the platelet count is $>1,500 \times 10^9/L$, interferon therapy may be required. Similarly, in those on preexisting cytoreductive therapy, only IFN α is felt to be safe during pregnancy. No drug is actually approved or licensed for use

during pregnancy, but the risk profile in high-risk patients is typically felt to be acceptable with the use of IFN α , whereas HU, anagrelide, and ruxolitinib are either known or suspected teratogens.

KEY POINTS

- A diagnosis of ET requires exclusion of reactive causes, as well as other myeloid neoplasm mimics.
- JAK2, CALR, or MPL mutations are present in 80% to 90% of ET patients; their presence proves the existence of a clonal myeloid disorder, but these mutations are not specific for ET, and their absence does not exclude a diagnosis of ET.
- Vascular risk classification is based on age, thrombosis history, and mutational status.
- Life expectancy is longer compared to those with other MPNs, but patients are at risk for ET-related morbidity and mortality over time, due to symptoms, vascular disturbance, and transformation.
- Antiplatelet therapy is used selectively in ET; cytoreductive agents such as HU, IFN, or anagrelide are options for higher-risk patients, or those with uncontrolled symptoms.

Myelofibrosis (prefibrotic, overt primary, and post-ET/PV)

CLINICAL CASE

A 71-year-old man with a history of prostate cancer, status post prostatectomy, gout, and cholecystitis presented with early satiety, left upper quadrant pain, and night sweats. Physical examination revealed an enlarged spleen (15 cm below the left subcostal margin). Leukocytosis (WBCs = $21 \times 10^9/L$), normocytic anemia (Hgb = 9.4 g/dL), and a normal platelet count ($292 \times 10^9/L$) were noted. Review of the peripheral blood smear revealed circulating blasts, teardrop cells, nucleated red blood cells, and immature WBCs (myelocytes and metamyelocytes). A bone marrow biopsy was hypercellular with megakaryocytic hyperplasia and atypia, marrow blasts of 4%, and grade MF-2 reticulin fibrosis. JAK2-V617F was detected with an allele burden of 34%. Metaphase cytogenetics showed 46,XY,del(13q) in all 20 metaphases examined. An ASXL1 mutation was also noted.

Introduction

MF includes prefibrotic myelofibrosis, overt/fibrotic primary MF, and MF that evolved from ET or PV (post-ETMF and post-PVMF). The annual incidence of PMF has been reported at between 0.2 to 0.5 cases per 100,000

persons per year. The natural history of MF is quite variable depending on the presence or absence of poor prognostic features. The median age at diagnosis of PMF is ~65 years, with 70% of cases diagnosed after 60 years of age and approximately 10% of cases diagnosed at <45 years of age.

Diagnostic criteria

The 2016 WHO revisions to MF diagnostic criteria include incorporation of CALR as a representative molecular marker and explicit mention of the importance of distin-

Table 18-11 Diagnostic criteria for prefibrotic PMF

Criteria	
Major	<ol style="list-style-type: none"> Megakaryocyte proliferation and atypia, without reticulin fibrosis >grade 1, with increased age-adjusted cellularity, granulocytic proliferation, and often decreased erythropoiesis Not meeting WHO criteria for CML, PV, ET, MDS, or other myeloid neoplasm Presence of a clonal marker, such as JAK2, CALR, or MPL mutations; in the absence, presence of another marker (ASXL1, EZH2, TET2, IDH, SRSF2, SF3B1), or absence of reactive causes of bone marrow fibrosis
Minor	<ol style="list-style-type: none"> Anemia Leukocytes $\geq 11 \times 10^9/L$ Palpable splenomegaly LDH above reference range
Diagnosis	Diagnosis requires meeting all three major criteria and at least one minor criterion

Adapted from Arber DA et al, *Blood*. 2016;127(20):2391–2405.

Table 18-12 Diagnostic criteria for overt PMF

Criteria	
Major	<ol style="list-style-type: none"> Megakaryocyte proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3. Not meeting WHO criteria for PV, ET, CML, MDS, or another myeloid neoplasm Presence of JAK2, CALR, or MPL mutations; in their absence, presence of another clonal marker (ASXL1, EZH2, TET2, IDH, SRSF2, SF3B1); or absence of reactive fibrosis
Minor	<ol style="list-style-type: none"> Anemia Leukocytes $\geq 11 \times 10^9/L$ Palpable splenomegaly Increased LDH Leukoerythroblastosis
Diagnosis	Diagnosis requires meeting all three major criteria and at least 1 minor criterion

Adapted from Arber DA et al, *Blood*. 2016;127(20):2391–2405.

Table 18-13 Differential diagnosis of PMF

Acute panmyelosis with myelofibrosis
MDS with fibrosis
Late-stage PV, ET, or CML with evolution to myelofibrosis
Hairy cell leukemia
Hodgkin lymphoma
Non-Hodgkin lymphoma
Plasma cell dyscrasias
Acute lymphoblastic leukemia
Metastatic carcinoma
Multiple myeloma
Chronic myelomonocytic leukemia
Systemic mastocytosis
Eosinophilic leukemia
Granulomatous infections (tuberculosis, histoplasmosis)
Renal osteodystrophy
Autoimmune MF

guishing prefibrotic MF from ET (Tables 18-11 and 18-12). Bone marrow histology, including megakaryocyte atypia, and minor clinical criteria should be helpful in distinguishing prefibrotic MF from ET. In a comparison of pre-PMF and overt PMF, the latter has more extensive reticulin fibrosis and, often, leukoerythroblastosis. With increasing degrees of fibrosis, a diagnostic marrow aspirate often is unobtainable, yielding a “dry tap.” In both cases, a driver mutation is identified in nearly 90% of cases; when negative, other clonal markers may be identified, satisfying this criterion. Diagnostic criteria for post-ETMF and post-PVMF appear in Table 18-6. As with other MPNs, it is important to exclude mimicking entities. These other malignant and nonmalignant causes of marrow fibrosis are listed in Table 18-13.

Blood and bone marrow features

As with ET and PV, MF patients can have leukocytosis and thrombocytosis. Unlike ET and PV, MF patients can have anemia, thrombocytopenia, and/or leukopenia. Because of the high cell turnover, LDH, bilirubin, and uric acid levels are commonly increased. Haptoglobin levels may be decreased, and there may be other clinical and laboratory indicators of low-grade hemolysis. In both pre-PMF and overt MF, an increase in atypical megakaryocytes should be present in the marrow. These megakaryocytes often cluster (Figure 18-5) and may have hyperchromatic or irregularly folded nuclei. Progressive fibrosis is characterized by accumulation of extracellular reticulin fibers (revealed

by silver staining) and collagen (revealed by trichrome staining). In advanced stages of PMF, the hematopoietic space may become completely replaced by fibroblasts and extracellular matrix material. Osteosclerosis may develop in some cases.

Proliferation of fibroblasts and other mesenchymal cells leading to bone marrow fibrosis has been linked to inflammatory response cytokines and megakaryocyte- and monocyte-derived growth factors, including platelet-derived growth factor (PDGF), basic fibroblast growth factor, and transforming growth factor beta, which also contributes to the stromal reaction. Elevated levels of IL-1 and tumor necrosis factor alpha are associated with augmented production or release of PDGF, basic fibroblast growth factor, and angiogenic factors such as vascular endothelial growth factor. Another unique feature of MF includes egress of circulating CD34⁺ cells, which can be 50-fold higher than in PV or ET. Higher levels of circulating CD34⁺ cells in PMF are associated with more advanced bone marrow fibrosis. This egress partially explains the presence of EMH, which can be observed in the liver and spleen, as well as the vertebral column (paraspinal or intraspinal lesions, which can lead to cord compression), lung (which can associate with pulmonary hypertension), pleura, retroperitoneum, eye, kidney, bladder, mesentery, and skin.

Disease course and prognosis

In general, MF patients are more symptomatic than those with ET or PV. Such symptoms are hypercatabolic in nature, including fever, night sweats, or weight loss. Fatigue can be quite pronounced. Pruritus and bone and muscle pain also occur. Splenomegaly-associated symptoms are common, including pain/abdominal discomfort and early satiety. Portal hypertension with ascites can complicate the disease course and can arise from portal vein thrombosis, EMH of the liver, or increased blood volume in the setting of massive splenomegaly. As above, consequences from nonhepatosplenic EMH are also observed. Pulmonary hypertension can occur and is often underrecognized. Anemia is also common, as nearly 75% will have a hemoglobin value less than normal, with approximately 50% having hemoglobin value of <10 g/dL and 25% being red cell transfusion dependent. Anemia is multifactorial, including from ineffective erythropoiesis, inflammatory iron sequestration, splenic sequestration, autoimmune hemolysis, myelosuppression from medication, or bleeding from portal HTN. These symptoms have a major influence on therapeutic planning, because for most, palliation of the major symptoms that impact quality of life is the goal of therapy.

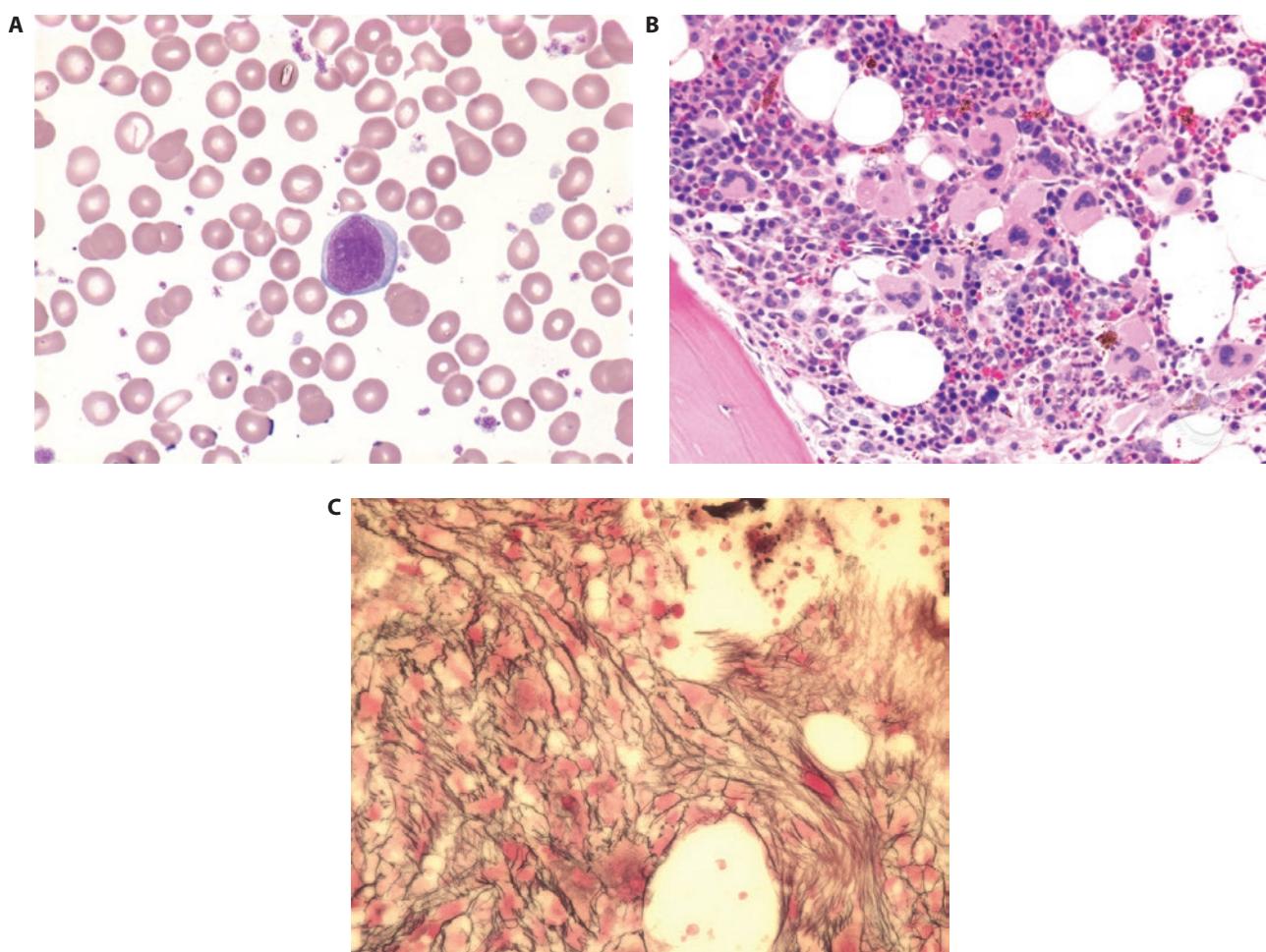


Figure 18-5 MF blood smear and bone marrow findings. (A) Blasts circulating in the peripheral blood can be found. (B) Megakaryocytic clustering is shown. The megakaryocytes are of variable size and show dysplastic nuclear changes. This finding was noted in a patient with a diagnosis primary myelofibrosis. (C) Marked reticulin fibrosis is demonstrated by a silver stain. Source: ASH Image Bank.

Most patients die while the disease remains in the MF phase. Causes of death stem from cardiovascular complications/thrombosis, bleeding, often in the setting of portal HTN, bone marrow failure, infection, and deterioration in the setting of an unrelated illness such as infection. Progression to MPN-BP is a cause of death in up to 30% of patients. Some MF patients progress due to complications from prior treatment, akin to therapy-related MDS.

Prognosis

Prognostic scoring systems continue to evolve and are mainly used for research studies, clinical trial selection, and as one tool to help identify those in need of referral to stem cell transplant. The clinical and laboratory features that predict a more aggressive disease course and shorter survival are summarized in Tables 18-14 and 18-15.

The presence of advanced age, constitutional symptoms, anemia ($\text{Hgb} < 10 \text{ g/dL}$), leukocytosis ($> 25 \times 10^9/\text{L}$), and circulating blasts ($\geq 1\%$) were found to contribute to poor outcomes and became the basis for the development of the International Prognostic Scoring System (IPSS). This score was designed primarily to evaluate prognosis at the time of original diagnosis. The Dynamic International Prognostic Scoring System (DIPSS) accounts for acquisition of additional risk factors with time. The same factors were considered in both scoring systems except that hemoglobin $< 10 \text{ g/dL}$ was given a higher score (2 points) compared with other risk factors in the IPSS. The next iteration, DIPSS-Plus, added transfusion dependence, thrombocytopenia ($< 100 \times 10^9/\text{L}$), and unfavorable karyotype to further refine prognosis.

In the molecular era, it has become clear that the type of driver mutation influences MF prognosis. First,

Table 18-14 IPSS-derived prognostic scoring systems used in PMF

Risk factor	IPSS (no. of points)	DIPSS (no. of points)	DIPSS-Plus (no. of points)
Age >65 years	1	1	DIPSS low=0
Constitutional symptoms*	1	1	DIPSS Int-1=1
Hgb <10 g/dL	1	2	DIPSS Int-2=2
WBC count >25 × 10 ⁹ /L	1	1	
Blood blasts ≥1%	1	1	DIPSS-high = 3
RBC transfusion dependence	—		1
Thrombocytopenia (<100 × 10 ⁹ /L)	—		1
Unfavorable karyotype†	—		1
Risk group	Points/median survival	Points/median survival	Points/median survival
Low	0: 11.3 years	0: NR	0: 15.4 years
Intermediate-1	1: 7.9 years	1 to 2: 14.2 years	1: 6.5 years
Intermediate-2	2: 4.4 years	3 to 4: 4 years	2 to 3: 2.9 years
High	≥3: 2.3 years	5 to 6: 1.5 years	4 to 6: 1.3 years

Data from Cervantes F et al, *Blood*. 2009;113:2895–2901; Passamonti F et al, *Blood*. 2010;115:1703–1708; and Gangat N et al, *J Clin Oncol*. 2011;29:392–397.

NR, not reached; RBC, red blood cell.

*Constitutional symptoms include fever, night sweats, weight loss >10% from baseline on the year prior to diagnosis.

†Unfavorable karyotype includes complex karyotype, one or two abnormalities that includes +8, -7/7q-, i(17q), -5/-5q, 12p-, inv(3), 11q23 rearrangement.

Table 18-15 MIPSS70 scoring systems

Risk factor	MIPSS70 (no. of points)	MIPSS70plus (no. of points)	MYSEC-PM* (no. of points) (also includes age)
Constitutional symptoms	1	1	1
Hgb <10 g/dL	1	1	2 (Hgb <11 g/dL)
WBC count >25 × 10 ⁹ /L	2	—	—
Thrombocytopenia (<100 × 10 ⁹ /L)	2	—	1 (<150 × 10 ⁹ /L)
Blood blasts ≥2%	1	1	2 (≥3% blasts)
Fibrosis grade ≥2	1	—	—
Absence of <i>CALR</i> type 1 mutation	1	2	2
Presence of HMR mutation	1	1	—
≥2 HMR mutations	2	2	—
Unfavorable karyotype†	—	3	—
Risk group	Points/5-year OS	Points/5-year OS	Median survival
Low	0 to 1: 95%	0 to 2: 91%	Low: NR
Intermediate	2 to 4: 70%	3: 66%	Int-1: 9.3 years
High	≥ 5: 29%	4–6: 42%	Int-2: 4.4 years
Very high	—	≥ 7: 7%	High: 2 years

Adapted from Guglielmelli P et al, *J Clin Oncol*. 2018;36(4):310–318; Passamonti F et al, *Leukemia*. 2017;31(12):2726–2731.

†Unfavorable karyotype includes any abnormal karyotype other than normal, sole abnormalities of 20q-, 13q-, +9, chromosome 1 translocation/duplication, -Y, or sex chromosome abnormality other than -Y.

HMR mutations: *IDH1/2*, *EZH2*, *ASXL1*, *SRSF2*.

*Also assigns 0.15 points to any age.

Calculators are available in both cases to predict prognosis.

NR, not reached.

prognosis is improved in those with *CALR* mutations, compared to other driver mutations; the prognosis was intermediate in those with *JAK2* or *MPL* mutations and the most concerning in patients lacking any driver mutation (“triple negative”). Even more nuanced, it has been suggested that the favorable prognosis associated with *CALR* mutations is restricted to those with type 1 mutations (52-bp deletions) or type-1 like based on modeling of the mutation.

Second, studies have demonstrated a negative impact of high-molecular risk (HMR) mutations (*IDH1/2*, *EZH2*, *ASXL1*, and *SRSF2*) on leukemia-free and overall survival, especially when more than two are present. The next iteration of an MF prognostic scoring system incorporates these molecular risk factors, along with clinical, laboratory, and histological features, in patients of a potential transplant age (MIPSS70, MIPSS70plus). Traditionally, these systems have been derived via study of PMF patients, but they have been used on those with post-ET and post-PV MF. However, other novel prognostic systems for these patients have been developed. Relevant variables here include age,

constitutional symptoms, anemia, thrombocytopenia, and a *CALR*-unmutated genotype.

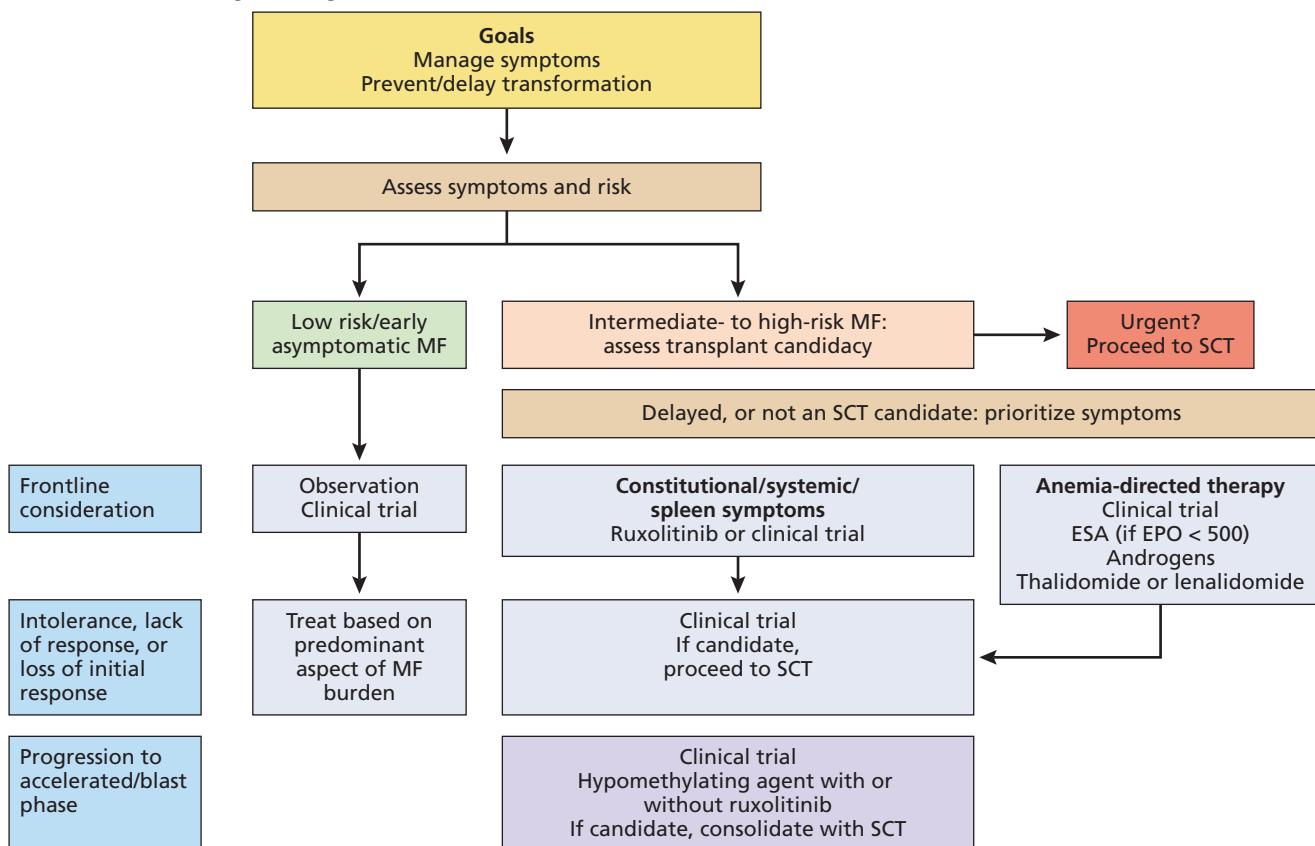
Management

The management of MF is influenced by risk and symptom burden. Risk classification is one tool that may aid in selection of stem cell transplant candidates. Nontransplant management is based on prioritizing and treating key determinants of the MF symptom burden, including from cytokine-related/systemic symptoms (fatigue, fever, weight loss, night sweats, pruritus, and bone pain) splenomegaly-related symptoms, and/or symptoms due to cytopenias (Figure 18-6).

Observation

Given the variable prognosis and presentation in MF, the initial diagnosis does not necessarily lead to a therapeutic intervention. In some cases, cytoreduction is indicated for thrombocytosis; as an example, while the prognosis differs, prefibrotic MF is treated in a similar manner as in ET. In the setting of post-ET/PV MF, the pace of disease and

Figure 18-6 MF management algorithm.



burden the patient experiences is variable, and in the absence of worsening disease burden (ie., worsening symptoms, problematic splenomegaly, increasing blasts, problematic cytopenias), patients might be best remaining on their preexisting ET/PV therapy until the MF declares itself to be more problematic. In those with asymptomatic, low-risk PMF and likely low-risk molecular features, a case can be made for observation. Experimental trials in low-risk PMF and slowly progressive post-ET/PV MF with a goal of delaying disease progression would be reasonable and are being considered with pegylated IFN or other agents, which might slow disease progression.

Stem cell transplantation

Transplant is a consideration, given the possibility of definitively addressing the disease burden. However, this high-risk, high-reward procedure requires careful consideration. Unique challenges include marrow fibrosis and marked splenomegaly, yet neither serve as an absolute contraindication. Marrow fibrosis is not permanent and can recede over time with a successful transplant. While splenomegaly may delay engraftment, in general, there is no indication for splenectomy prior to transplant, given its morbidity and mortality. Of course, additional challenges intrinsic to the transplant procedure include toxicity from the conditioning regimen, graft failure, graft-versus-host disease, and relapse. Outcomes vary, derived from heterogeneous but selected series; 5-year overall survival roughly ranges from 30% to 65%.

Selection of the ideal candidate remains challenging, and age/comorbidities, caregiver availability, type of donor, and an individual's own risk philosophy factor into decision making.

As above, prognostic scoring systems continue to evolve and serve as one tool to identify potential candidates. Transplant is considered in those with intermediate- to high-risk MF; the risk of transplantation probably outweighs the benefits in those with low-risk disease. However, there is increasing consideration of the molecular profile; because the absence of *CALR* and presence of HMR mutations negatively impact prognosis, patients with intermediate-1-risk disease but an adverse molecular profile may be considered for transplant.

Novel pretransplant strategies, including the use of JAK inhibition, are under investigation. In the contemporary era, many referred patients will have been treated with JAK inhibitors prior to transplant. The rationale includes improving performance status and decreasing splenomegaly; further, there is speculation that modulating the cytokine profile could influence graft-versus-host disease risk. If JAK inhibition is used pretransplant, questions regarding dura-

tion and best approach for weaning JAK inhibition prior to transplant or overlapping it with a conditioning regimen remain. Pretransplant treatments may also include iron chelation as well as cytoreduction in individuals who have an increased blast percentage in the blood or bone marrow.

Symptom-directed management

Predominant cytokine-associated and/or splenomegaly-related symptoms

JAK inhibition. Regardless of the type of driver mutation, JAK-STAT dysregulation is a central pathogenic mechanism in MF, and the basis for the development of JAK inhibitors. Accordingly, mutational testing is not necessary to guide use of JAK inhibitor therapy. Rather, the symptom profile is the important consideration; these agents are not advised for asymptomatic patients. Beyond symptoms, baseline platelet counts should be considered, as well as risk grouping because trials have typically included those with intermediate risk and beyond.

Ruxolitinib, a JAK1/JAK2 inhibitor, was the first pharmacologic agent to be FDA approved in MF. The pivotal phase 3, multicenter, double-blind, placebo-controlled, randomized trial Controlled Myelofibrosis Study with Oral JAK Inhibitor Treatment (COMFORT-I) showed at least a 35% spleen size reduction assessed by radiologic imaging (magnetic resonance imaging or computed tomography [CT] scan) at 24 weeks in 41.9% of patients in the ruxolitinib arm compared with just 0.7% in the placebo. Furthermore, decreases in the total symptom score by >50% at 24 weeks were noted in the ruxolitinib-treated patients (45.9%) compared with those receiving placebo (5.3%). A European counterpart, COMFORT-II, compared ruxolitinib versus best available therapy (BAT) and demonstrated a spleen volume reduction of at least 35% in 28% of patients on ruxolitinib compared with 0% in patients on BAT at 48 weeks. Similarly, quality-of-life measures and disease-related symptoms were better in the ruxolitinib-treated patients. Five-year follow-up reported a median duration of spleen response of about 3 years. The impact on mutational allele burden has been modest (of unknown significance), as have been histological changes. However, survival benefits have been reported, likely due to changes in performance/functional status, rather than through achievement of complete or partial remission.

The main side effects of ruxolitinib include dose-dependent anemia and thrombocytopenia, and therefore these baseline parameters influence dosing. Additional side effects can include headache, bruising, dizziness, diarrhea, weight gain, and increase in cholesterol. Skin cancers and

infections (typical and atypical) have also been reported. Additional JAK inhibitors (including pacritinib, fedratinib, and NS-018) are in development in hopes of offering similar or improved clinical efficacy, with potentially less myelosuppression. Multiple combination strategies for use with ruxolitinib have also been tested or are ongoing with a hope of demonstrating synergistic effects or offsetting/ameliorating myelosuppression or cytopenias.

Splenectomy and splenic radiation. Splenectomy is associated with a high morbidity and mortality, attributed to abdominal thrombotic events, postoperative hemorrhage, and postsplenectomy leukocytosis and thrombocytosis. The advent of JAK inhibitor therapy and its significant impact on splenomegaly have decreased the need and consideration for splenectomy. However, splenectomy can be considered as a salvage option for those individuals who have failed JAK inhibitor therapy and/or other medical therapies. Splenic radiation can offer temporary symptomatic relief, but it can be associated with prolonged and serious myelosuppression. This procedure would also be reserved for nonsurgical candidates, refractory to medical therapies, without clinical trial options, but patients and radiation oncologists must be aware of the risk for high-grade cytopenias, and only low-dose radiation should be considered.

Extramedullary hematopoiesis (EMH) can also involve other sites, including the vertebral column (paraspinal or intraspinal lesions, which can lead to cord compression), lung (which can associate with pulmonary hypertension), pleura, retroperitoneum, eye, kidney, bladder, mesentery, and skin. Consultation with radiation oncology may be required in some cases.

Cytopenia-directed therapy

Anemia represents a currently unmet treatment need, though conventional options have been utilized. These include erythropoiesis-stimulating agents (ESAs), which can occasionally improve anemia in non-transfusion-dependent patients with EPO levels <500 mIU/mL and especially <125 mIU/mL. Splenomegaly may worsen during ESA therapy. Androgens, including danazol, oxymetholone, nandrolone, and testosterone, can lead to anemia or platelet responses in 10% to 35% of patients. A small subset of patients with evidence of hemolytic anemias can respond to corticosteroids. Immunomodulatory drugs also can have an impact on myelofibrosis-associated anemia, perhaps from impact on the intramedullary cytokine milieu, which may be inhibiting hematopoiesis. Such options include thalidomide/tapering prednisone or le-

nalidomide/tapering prednisone. Thrombocytopenia responses have been reported with thalidomide/prednisone. Toxicities can include sedation and neuropathy. Lenalidomide can have activity in MF-associated anemia, particularly in those with a deletion 5q, but can be myelosuppressive. A randomized clinical trial of pomalidomide did not show benefit over placebo.

MPN-blast phase

MPN-BP is typically refractory to induction chemotherapy and portends a poor prognosis. This refractory nature highlights the importance of consideration of stem cell transplant earlier in the course of the disease in individuals with high-risk features. While approximately 40% to 50% of patients with MPN-BP treated with AML-like induction chemotherapy may return to a more chronic phase of an MPN, the duration is usually short. In this setting, if stem cell transplantation can be performed, it should occur in a rapid fashion. In general, MPN-BP represents an appropriate indication for clinical trial referral, and induction chemotherapy should be only considered in stem cell transplant candidates. Use of hypomethylating agents, with or without JAK inhibition, is an additional and increasingly utilized option, whether or not patients will receive stem cell transplant as a means of consolidation.

KEY POINTS

- The spectrum includes prefibrotic MF, overt primary MF, and post-ET/PV MF.
- Most are diagnosed in the seventh decade and beyond, and the prognosis can be quite variable, depending on clinical, laboratory, and molecular findings.
- The majority of patients develop anemia, splenomegaly, and significant symptoms during the course of their disease.
- Therapeutic approaches are guided by risk and symptom burden; stem cell transplantation is an important consideration in selected higher-risk patients.
- Therapy with JAK inhibition has been very impactful by decreasing splenomegaly, improving MF-related symptoms, and decreasing disease-associated morbidity and mortality.
- Splenectomy can be considered for palliation in those refractory to medical therapies but is employed less frequently in the JAK inhibitor era.
- Anemia-directed therapies include ESAs, androgens, and immunomodulatory drugs.

Other *BCR-ABL1*-negative MPNs

Chronic neutrophilic leukemia

CLINICAL CASE

A 64-year-old, previously healthy executive noticed a change in her abdominal girth for about 3 months. This was accompanied by a feeling of bloatedness, early satiety, occasional nausea, and intermittent episodes of itching. She decided to have a routine blood test at a local clinic and was found to have the following CBC results: WBCs = $27 \times 10^9/L$, Hgb = 12.9 g/dL, hematocrit = 40%, mean corpuscular volume (MCV) = 84 fL, platelet count = $315 \times 10^9/L$, and absolute neutrophil count (ANC) = $25 \times 10^9/L$; occasional metamyelocytes and myelocytes were noted but accounting for 5% of WBCs, and no myeloblasts were seen. She was referred to a hematologist who noted hepatosplenomegaly and mild cervical lymphadenopathy by physical examination. A bone marrow aspiration and biopsy were performed, showing increased numbers of neutrophilic granulocytes, a hypercellular marrow (95%), no dysplastic changes, and 3% myeloblasts. Metaphase cytogenetics showed 46, XX [20]. Molecular testing or FISH for *BCR-ABL1*, *PDGFRA*, *PDGRB*, *FGFR1*, *PCM1-JAK2*, and *JAK2 V617F* were all unremarkable. Subsequently, her physician sent peripheral blood for *CSF3R* mutation testing, which returned positive for T618I.

CNL is a very rare chronic MPN recognized as a distinct entity by the 2016 WHO classification. CNL, historically, has been a challenging diagnosis to make, requiring exclusion of reactive neutrophilia and other myeloid malignancies, including typical and atypical CML, as well as chronic myelomonocytic leukemia (CMML). The incidence and prevalence of CNL is difficult to estimate, and males and females appear to be equally affected. CNL occurs more commonly in older patients (often in the seventh decade), though adolescent cases have been described.

Diagnosis

Although some patients have an incidental discovery of leukocytosis, others present with fatigue and constitutional symptoms, such weight loss and night sweats. Splenomegaly is the most frequently found clinical feature in patients with CNL. Some patients will present with gastrointestinal tract bleeding, thrombocytopenia, pruritus, and gout. Transformation to acute leukemia has been reported.

CNL is defined by the WHO as having a sustained, nonreactive leukocytosis $>25 \times 10^9/L$, with $>80\%$ segmented/band neutrophils, $<10\%$ immature granulocytes, $<1 \times 10^9/L$ monocytes, and $<1\%$ blasts in the peripheral

blood. Bone marrow biopsy demonstrates hypercellularity with a striking neutrophil proliferation with a myeloid-to-erythroid ratio reaching up to 20:1. Blasts or promyelocytes are not increased in number; dysplasia and reticulin fibrosis are not evident. Other MPNs should be excluded, and there should be no evidence of *BCR-ABL1*, *PDGFRA*/ *PDGFRB*, *FGFR1*, or *PCM1-JAK2* mutations. The presence of the *CSF3R* T618I mutation, or other activating mutations, has become part of the diagnostic criteria.

Course and prognosis

The clinical course of CNL is heterogeneous. Disease acceleration often manifests with the development of progressive neutrophilia with resistance to previously effective therapy, progressive splenomegaly, or worsening thrombocytopenia, or with cytogenetic clonal evolution. Transformation to blast phase (AML) was reported to occur in a significant proportion of patients at a median of 21 months from diagnosis. Progressive neutrophilia associated with anemia and thrombocytopenia have been reported, as has transformation to myelodysplasia. Although CNL is regarded as a relatively slowly progressive disease with survival ranging from 6 months to >20 years, one retrospective analysis of 40 patients with CNL reported a median survival time of 23.5 months. Most common causes of death included intracranial hemorrhage ($N=9$), progressive disease ($N=5$), blastic transformation ($N=4$), infection ($N=1$), and treatment-related complications ($N=1$).

Management

Optimal treatment for patients with CNL remains to be defined. Splenectomy has resulted in worsening of neutrophilic leukocytosis and is not routinely recommended. Treatment of CNL, to date, has consisted largely of cytoreductive agents, such as HU, where clinical responses occur, but lack durability. Similar to other chronic MPNs, interferons (ie., IFN α) have been used. Allogeneic hematopoietic cell transplantation can be curative, but is usually reserved for patients with accelerated or blastic transformation. Given the potential for blastic transformation and progressive refractory neutrophilia, however, allogeneic hematopoietic cell transplantation (HCT) may be appropriate for younger patients. The use of tyrosine kinase inhibitors in the treatment of CNL is intriguing, but not yet confirmed with clinical data. In the first report of *CSF3R* mutations in CNL, Maxson et al described a single patient with a membrane proximal mutation (*CSF3R* T618I) and improvement in neutrophilic leukocytosis and thrombocytopenia when treated with ruxolitinib. In another report, a patient with a membrane proximal mutation (also

CSF3R T618I) and a *SETBP1* mutation was refractory to ruxolitinib and HU. The safety and efficacy of ruxolitinib in CNL (and atypical CML) are currently under investigation in a multicenter study (clinical trials identifier: NCT02092324). No reports have been published detailing the clinical utility of dasatinib in CNL or atypical CML harboring truncation mutations in *CSF3R*.

Chronic eosinophilic leukemia, not otherwise specified

CLINICAL CASE



A 35-year-old male graduate student came to the university health clinic because of nonproductive cough, diarrhea, fatigue, intermittent fevers (102°F [38.9°C]), and muscle aches. He initially attributed these symptoms to stress, but sought medical attention due to persistence over a 2-month period. A CBC showed the following: WBCs = $19 \times 10^9/L$, Hgb = 11.5 g/dL, MCV = 83 fL, platelets = $188 \times 10^9/L$, ANC = $12 \times 10^9/L$, and absolute eosinophil count (AEC) = $3.4 \times 10^9/L$. There were 3% circulating blasts in the peripheral blood. Workup for connective tissue diseases, parasitic infections, and allergies was unremarkable. His subsequent evaluation by a hematologist confirmed an eosinophilic leukocytosis, and a bone marrow aspiration and biopsy showed 6% bone marrow blasts with no dysplastic changes. Metaphase cytogenetics were normal (46,XY [20]). He had no abnormalities in *PDGFRA*, *PDGFRB*, *FGFR1*, *PCM1-JAK2*, or *BCR-ABL1*.

Chronic eosinophilic leukemia (CEL) is characterized by an autonomous, clonal proliferation of eosinophil precursors resulting in persistent elevation of eosinophils in the peripheral blood, bone marrow, and peripheral tissues. Although CEL, not otherwise specified (CEL-NOS), is a rare MPN, the true incidence of these neoplasms is unknown. Nonetheless, myeloproliferative eosinophilic syndromes seem to occur much more often in men than in women. The peak incidence is in the fourth decade, but CEL-NOS can occur at any age, including childhood.

Clinical features and diagnostic criteria

A minority of CEL-NOS patients are identified incidentally, and more commonly, patients present with fever, fatigue, cough, pruritus, diarrhea, angioedema, and muscle pain. End-organ damage can be a manifestation of a direct eosinophilic infiltrate or secondary to the release of cytokines and the contents of toxic granules. The most serious clinical findings relate to endomyocardial fibrosis resulting from eosinophilic infiltration of the heart, leading to con-

strictive pericarditis, fibroblastic endocarditis, myocarditis, or intramural thrombus formation (due to scarring of the mitral or tricuspid valves). Peripheral and central nervous system findings can include mononeuritis multiplex, peripheral neuropathy, and paraparesis, as well as cerebellar involvement, epilepsy, dementia, cerebral infarction, and eosinophilic meningitis. Pulmonary involvement includes idiopathic infiltrates, fibrosis, pulmonary effusions, and pulmonary emboli. Skin manifestations are common and can take many forms, including angioedema, urticaria, papulo-nodular lesions, and erythematous plaques. Gastrointestinal involvement by eosinophilia can result in ascites, diarrhea, gastritis, colitis, pancreatitis, cholangitis, or hepatitis.

The WHO criteria for diagnosis of CEL-NOS require the presence of eosinophilia ($1.5 \times 10^9/L$); a clonal cytogenetic or molecular abnormality or blasts cells >2% in the peripheral blood or >5% in the bone marrow; lack of *BCR-ABL1*, *PDGFRA/PDGFRB*, *FGFR1*, or *PCM1-JAK2* rearrangements; bone marrow blasts <20%; and the absence of inv(16)(p13.1q22). Consideration for idiopathic Hypereosinophilic syndrome (HES) requires exclusion of patients with infectious, allergic, autoimmune, or collagen vascular disorders or pulmonary or neoplastic conditions (including clonal lymphoid disorders), which are known to be associated with secondary eosinophilia. Idiopathic HES is therefore classified in patients who have the following characteristics: (1) persistent eosinophilia ($>1.5 \times 10^9/L$) lasting for at least 6 months (though this is in evolution, as treatment needs can be urgent, and waiting 6 months is inappropriate); (2) no reactive causes of eosinophilia; (3) no associated clonal myeloid neoplasm like AML, MDS, MDS/MPN overlap, MPN, and systemic mastocytosis; (4) no cytokine-producing immunophenotypically aberrant T-cell population; (5) no increased myeloblasts in the peripheral blood or bone marrow; and (6) no evidence of eosinophil clonality and with end-organ damage. If the previous six criteria were fulfilled except that there is no end-organ damage, then its best classified as idiopathic hypereosinophilia. Panels using next-generation sequencing targeting genes commonly mutated in myeloid malignancies can be helpful, as once clonality is established, cases of HES can be redefined as CEL-NOS.

Course and prognosis

CEL-NOS typically carries a poor prognosis. Blast transformation can occur, and poor prognostic features include marked splenomegaly, cytogenetic abnormalities, and dysplastic myeloid features in the bone marrow. In a report on 10 patients with CEL-NOS, the median overall survival was 22 months, and one-half transformed to acute leukemia at a median of 20 months from the time of diagnosis.

Idiopathic HES can have a variable course and tends to be a chronic disorder. In one series, including patients with idiopathic HES and eosinophilic leukemia, 80% of patients were alive at 5 years after diagnosis, and 42% were alive at 15 years.

Management

Treatment is indicated for patients with evidence of end-organ damage. Therapy for CEL-NOS and idiopathic HES is aimed primarily at decreasing the eosinophil count, improving symptoms, and preventing end-organ damage or thromboembolic complications. Inadequate data exist to support initiation of therapy based on a specific eosinophil count in the absence of organ disease. Corticosteroids (eg, prednisone 1 mg/kg/d) have typically been the treatment of choice in HES to reduce eosinophil numbers and minimize the cytotoxic effects of the eosinophilic granules. Steroid-resistant patients traditionally have been treated with HU. IFN α can elicit sustained hematologic and cytogenetic remissions in idiopathic HES and CEL-NOS patients refractory to other therapies, including prednisone and HU. In one retrospective study where 46 patients were treated with IFN α , the response rates were 50% and 75% for monotherapy or in combination with steroids, respectively. Lack of steroid responsiveness, or failure of HU or IFN α , may warrant consideration of cytotoxic chemotherapeutic agents, such as vincristine, cyclophosphamide, or etoposide. Imatinib is also a consideration, but expectations regarding response rates and duration are much lower compared to those patients with *PDGFRA* or *PDGFRB* rearrangements.

Anti-IL-5 antibody approaches, such as mepolizumab, have been undertaken in HES based on the cytokine's role as a differentiation, activation, and survival factor for eosinophils. Long-term results have been presented in 78 patients who had a median exposure of 251 weeks. Ninety-seven percent of patients experienced adverse effects, but approximately one-third were considered drug related; cough, fatigue, headache, upper respiratory infections, and sinusitis were most commonly observed. Suppression of eosinophilia was noted, and in the first 4 months, the median prednisone dose decreased from 20 to 0 mg (1.8 mg was the median dose over the course of the study). Although it has regulatory approval for the treatment of certain subsets of asthma and eosinophilic granulomatosis with polyangiitis (EGPA), mepolizumab is not approved for CEL-NOS at the time of this writing, but it is available in a clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov) identifier: NCT02836496).

Use of the anti-CD52 monoclonal antibody alemtuzumab in refractory HES based on the expression of the CD52 antigen on eosinophils has also been reported. A

study that included 11 patients with HES and CEL used alemtuzumab in escalating doses of 5, 10, and 30 mg intravenously from days 1 to 3, then maintained at the tolerated dose 3 times per week for a total of 12 doses. This resulted in a 91% complete hematologic response after a median of 2 weeks. The median duration of response was 3 months. A second retrospective study of 12 patients with HES or CEL treated with alemtuzumab reported a complete hematological response in 10 (83%) for a median of 66 weeks. Eleven relapses were reported, and five achieved a second complete hematological response (CHR) with retreatment. Infusion reactions and viral infections including cytomegalovirus (CMV), zoster, and Epstein barr virus (EBV) were reported. Despite these results, the data on alemtuzumab remain limited and the drug is best considered an investigational therapy for this condition at this time.

Myeloproliferative neoplasm, unclassifiable

The term *MPN, unclassifiable* (MPN-U) should be used to describe only those patients who meet clinical, laboratory, and morphologic criteria of MPNs but who fail to present features of any single MPN entity or patients who present with overlapping features of two or more MPN entities. The demonstration of pathognomonic molecular abnormalities, such as *BCR-ABL1* fusion or the *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* rearrangements, excludes the diagnosis of MPN-U. In the era of molecular diagnostics, it is expected that the number of MPN-U will likely decrease. The exact incidence, median age at onset, and sex distribution of MPN-U are not truly known.

Clinical features

The clinical features of patients with MPN-U is variable, as this is a heterogeneous group of disorders. Patients can present with minimal to no organomegaly and well-preserved peripheral blood counts in the very early stages of the disease or massive organomegaly, extensive myelofibrosis, and severe cytopenias in advanced cases. Unexplained portal or splanchnic vein thrombosis may be the initial presenting feature in these patients.

Course and prognosis

The clinical course and prognosis for patients with MPN-U can be extremely heterogeneous. Patients with early-stage disease can safely be followed every 6 months and generally will develop features of unique MPN entities. Patients in whom unique MPN entities are no longer recognizable tend to have aggressive clinical courses and very poor prognosis.

Systemic mastocytosis

CLINICAL CASE

A 67-year-old retired woman has been experiencing fever, chills, diarrhea, a persistent urticaria-like rash, flushing, and palpitations for the past 5 months. She decided to see a primary care doctor who noticed palpable lymph nodes in the neck and axillary regions and a palpable spleen tip by physical examination. Routine blood work showed normocytic anemia ($Hgb = 10.1 \text{ g/dL}$, $MCV = 92 \text{ fL}$); leukocytosis ($WBCs = 25 \times 10^9/\text{L}$) with increased lymphocytes (40%), monocytes (28%), and eosinophils (12%); and mild thrombocytopenia (platelets = $97 \times 10^9/\text{L}$). Review of blood work 6 months prior showed similar CBC findings. The patient saw a hematologist and underwent a bone marrow aspiration and biopsy, which showed dysplastic changes in the erythroid and megakaryocytic lineages with 5% blasts in the bone marrow. The biopsy showed a hypercellular marrow with spindle-shaped mast cell infiltration grade of 50%. Flow cytometry of the bone marrow aspirate showed increased CD25 expression on mast cells. A *KITD816V* mutation also was identified. Metaphase cytogenetics showed 46, XX [20]. Total tryptase level was 450 ng/mL. The patient was diagnosed with systemic mastocytosis with an associated hematological neoplasm (SM-AHN), specifically chronic myelomonocytic leukemia (CMML-1).



disorders is poorly defined; SM is felt to be a very rare disease. Although mastocytosis can be diagnosed at any age, CM is more common in children, whereas SM occurs predominantly in adults. These disorders appear to have a slight male predominance.

Pathobiology

Mast cells are long-lived hematopoietic cells with unique biologic properties and a unique spectrum of mediators and cell surface antigens. Mature mast cells are best known for their involvement in allergic inflammation mediated by allergen-specific immunoglobulin E (IgE) and tend to reside in diverse organs, often in close vicinity to smaller or larger blood vessels. Mast cell survival depends largely on SCF; KIT is the protein TK receptor for SCF.

Other somatic mutations, including *TET2*, *DNMT3A*, *ASXL1*, *SF3B1*, and *CBL* mutations, also have been identified in a subset of mastocytosis patients, particularly in those with an associated hematological non-mast cell disease (SM-AHN). In a study of 39 *KIT D816V*-mutated SM patients, Schwaab et al reported that the presence of additional somatic mutations (most frequently *TET2*, *SRSF2*, *ASXL1*, *CBL*, and *RUNX1*) were more common in those with advanced SM and contributed to inferior survival (in particular, the S/A/R profile with mutation in *SRSF2*, *ASXL1*, and *RUNX1*). When SM is diagnosed in conjunction with another hematologic neoplasm (~30% to 40% of cases), the underlying neoplasm is typically of myeloid rather than lymphoid origin. Mutations in *KIT D816V* have been identified in both the mast cell and associated hematological non-mast cell lineage disease (AHNMD) compartment, which potentially may indicate a shared pathogenetic origin in a hematopoietic progenitor. Patients with indolent systemic mastocytosis (ISM) appear to have more of a pure *KIT D816V*-driven disease. Apart from organ infiltration, the consequences of mastocytosis stem from mediator release, as mast cells are activated and degranulate. Mast cells contain a variety of mediators, including histamine, inflammatory cytokines such as IL-3 and IL-16, and tumor necrosis factor. In addition, mast cells contain secretory granule proteases, most commonly tryptase, which is increased in mast cell diseases. An increase in tryptase levels serve as a minor criterion for diagnosis (unless AHNMD is present), and although the level itself cannot distinguish SM subgroups, marked increases are seen in more advanced/aggressive subtypes. Additionally, measurement serves as a practical means of assessing mast cell burden and monitoring response to therapy.

Clinical features and diagnosis

Clinical features at the time of presentation for patients with mastocytosis depend on the extent of organ infiltration,

Mastocytosis encompasses a heterogeneous spectrum of disorders characterized by mast cell proliferation and accumulation (Figure 18-7). Clinical manifestations of mast cell disorders are caused by uncontrolled proliferation of tissue mast cells and the release of mast cell-derived mediators. While cutaneous mastocytosis (CM) is usually a chronic, indolent disease, SM can be either indolent or more aggressive and even life threatening. Given that SM has a spectrum of clinicopathologic features in common with MPNs, the 2008 WHO classification included SM under the broader umbrella of MPNs. However, in 2016, it was moved to its own category. Mastocytosis can be classified according to clinicopathologic and laboratory findings (Table 18-16). Indolent SM usually has a low burden of disease, and smoldering SM is characterized by two or more B findings (Table 18-17). Advanced SM is an umbrella term for aggressive SM (ASM), SM-AHN, and mast cell leukemia (MCL). ASM is defined by one or more C findings (organ damage), and SM-AHN and MCL also usually exhibit C findings (Table 18-17). MCL is histopathologically defined as 20% or more neoplastic mast cells on an aspirate. In contrast to more indolent SM, advanced SM typically exhibits shortened survival usually requiring cytoreduction. The incidence of mastocytic

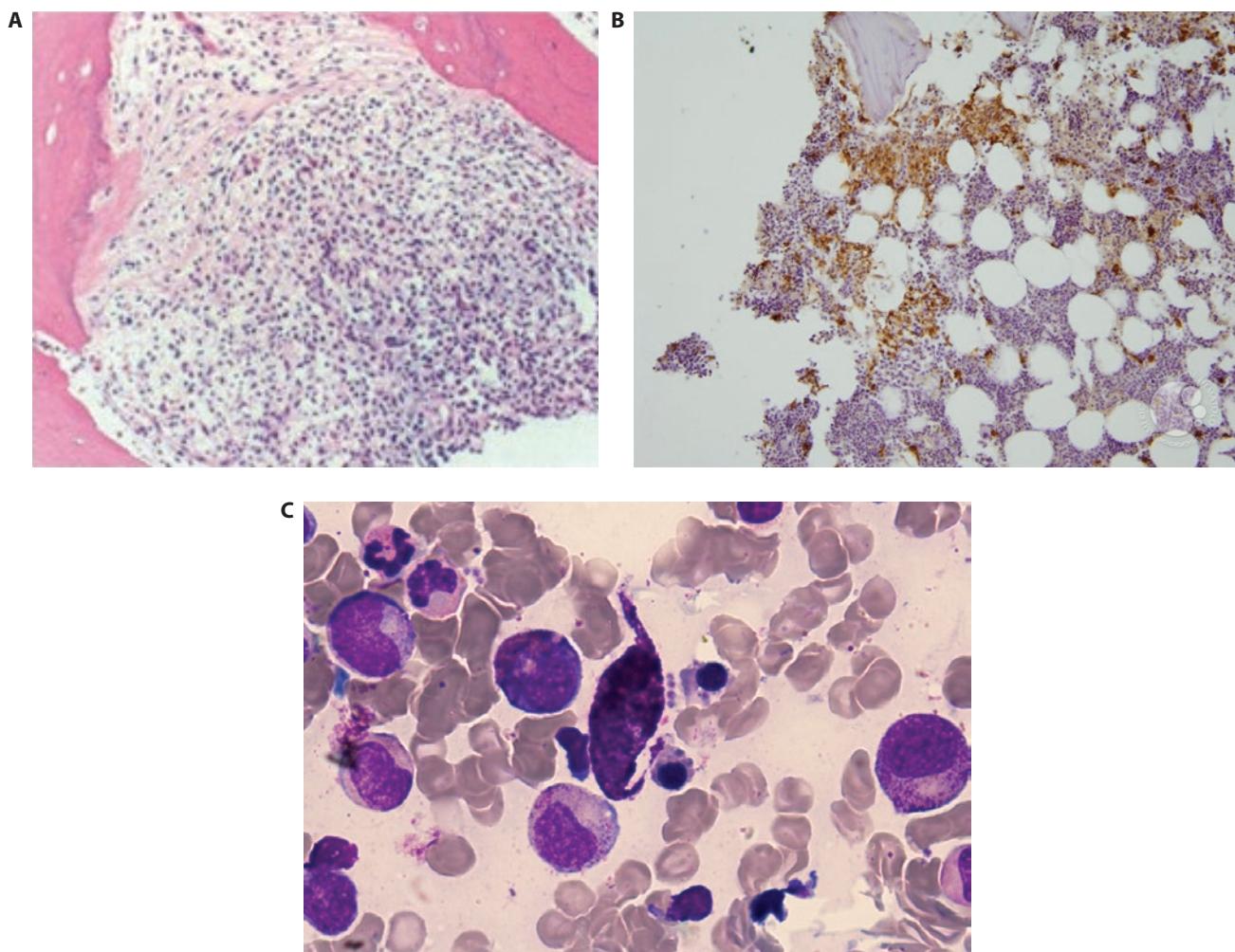


Figure 18-7 Bone marrow involvement with systemic mastocytosis. (A) A marrow biopsy shows areas of infiltration or complete replacement by elongated, spindle-shaped cells (hematoxylin-and-eosin stain; original magnification, 85 \times). Photo courtesy of Steven J. Kussick (University of Washington, Seattle, WA). (B) Mast cells stain positive (brown) for tryptase. Serum tryptase level was elevated at 45.9 ng/mL (200 \times). Source: ASH Image Bank/Ganesh Chandrasekhar Kudva and Leonard E. Grosso. (C) Spindle-shaped mast cells on a bone marrow aspirate. Source: ASH Image Bank/Sylvie Bouvier and Anne Arnaud.

Table 18-16 The 2016 WHO classification of mastocytosis

- 1. CM
- 2. Systemic mastocytosis
 - a. Indolent systemic mastocytosis*
 - b. Smoldering systemic mastocytosis*
 - c. SM-AHN
 - d. ASM*
 - e. MCL
- 3. Mast cell sarcoma

Adapted from Arber DA et al, *Blood*. 2016;127(20):2391–2405.

*These subtypes require information regarding B and C findings for complete diagnosis, all of which may not be available at the time of initial tissue diagnosis.

consequences of mediator release, and whether or not a non-mast cell disorder is also present. Cutaneous manifestations of mastocytosis typically include a reddish-brown maculopapular eruption (*urticaria pigmentosa*) or, less often, a diffuse erythema, plaques, or nodules. The classic description of urticaria following stroking of the skin is coined as the Darier sign. Telangiectasia macularis eruptiva perstans, characterized by red-brown macules with irregular borders and a telangiectasia-like appearance, is a less common cutaneous manifestation. Cutaneous manifestations may be the only consequence of mast cell disease in children. Blistering

Table 18-17 WHO criteria for diagnosis of cutaneous and systemic mastocytosis

Cutaneous mastocytosis	
Skin lesions demonstrating the typical clinical findings and typical infiltrates of mast cells in a multifocal or diffuse pattern in an adequate skin biopsy. Absence of features/criteria for the diagnosis of SM.	
Systemic mastocytosis	
The diagnosis of SM may be made if one major criterion and one minor criterion are present or if three minor criteria are fulfilled.	
<i>Major criterion</i>	
Multifocal, dense infiltrates of mast cells (≥ 15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organ(s).	
<i>Minor criteria</i>	
a. In biopsy sections of bone marrow or other extracutaneous organs, $>25\%$ of the mast cells in the infiltrate are spindle shaped or have atypical morphology or, of all mast cells in bone marrow aspirate smears, $>25\%$ are immature or atypical mast cells.	
b. Detection of <i>KIT</i> point mutation at codon 816 in bone marrow, blood, or other extracutaneous organ(s).	
c. Mast cells in bone marrow, blood, or other extracutaneous organs that coexpress CD117 with CD2 and/or CD25.	
d. Serum total tryptase persistently >20 ng/mL (unless there is an associated clonal myeloid disorder, in which case this parameter is not valid).	
Indolent systemic mastocytosis	
Meets criteria for SM.	
No evidence of an associated clonal hematologic non-mast cell lineage disease.	
No "C" findings.	
Mast cell burden is low, and skin lesions are almost invariably present.	
*Bone marrow mastocytosis: bone marrow involvement, but no skin lesions.	
*Smoldering systemic mastocytosis: with two or more "B" findings but no "C" findings.	
Aggressive systemic mastocytosis	
Meets criteria for SM.	
One or more "C" findings.	
No evidence of mast cell leukemia.	
*Lymphadenopathic mastocytosis with eosinophilia (provisional subvariant): progressive lymphadenopathy with peripheral blood eosinophilia, often with extensive bony involvement and hepatosplenomegaly, but usually without skin lesions. Exclude cases with rearranged <i>PDGFRA</i> .	
Systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease	
Meets criteria for SM.	
Associated clonal hematologic non-mast cell lineage disorder (MDS, MPN, AML, lymphoma, or other hematologic neoplasm that meets the criteria for a distinct entity in the WHO classification).	
Mast cell leukemia	
Meets criteria for SM.	
Diffuse bone marrow infiltration by atypical immature mast cells. Bone marrow aspirate contains $>20\%$ mast cells. Usually $>10\%$ circulating mast cells on peripheral blood.	
"B" findings	
1. Bone marrow biopsy showing $>30\%$ infiltration by mast cells (focal, dense aggregates) and/or serum total tryptase level >20 ng/mL.	
2. Signs of dysplasia or myeloproliferation in non-mast cell lineage, but insufficient criteria for definitive diagnosis of hematopoietic neoplasm by WHO, with normal or only slightly abnormal blood counts.	
3. Hepatomegaly without impairment of liver function, and/or palpable splenomegaly without hypersplenism, and/or palpable or visceral lymphadenopathy.	

Table continues on next page

Table 18-17 WHO criteria for diagnosis of cutaneous and systemic mastocytosis (*continued*)

“C” findings
1. Bone marrow dysfunction manifested by one or more cytopenia (ANC $<1 \times 10^9/L$, Hgb $<10\text{ g/dL}$, or platelets $<100 \times 10^9/L$), but no frank non-mast cell hematopoietic malignancy.
2. Palpable hepatomegaly with impairment of liver function, ascites, and/or portal hypertension.
3. Skeletal involvement with large-sized osteolysis and/or pathologic fractures.
4. Palpable splenomegaly with hypersplenism.
5. Malabsorption with weight loss due to gastrointestinal mast cell infiltrates.

Adapted from Horny HP, et al. In: Swerdlow SH, et al, eds. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. (Lyon, France: IARC Press; 2008).

can occur in pediatric patients and represents an aggressive form of urticaria pigmentosa.

Mastocytosis is typically systemic in adults and often includes bone marrow infiltration. Other organs commonly involved include the liver, spleen, lymph nodes, and gastrointestinal mucosa. Clinical features of SM are categorized in four distinct groups: (1) constitutional symptoms (eg., fatigue, fever, weight loss); (2) cutaneous manifestations, as described above; (3) systemic mediator-related symptoms (eg., abdominal pain or bloating, dyspepsia, diarrhea, flushing, headache, hypotension, anaphylaxis); and (4) musculoskeletal complaints (eg., bone pain and myalgias, osteopenia, fractures). Anaphylaxis after a Hymenoptera sting can also indicate underlying mastocytosis, and a workup for mast cell disease is warranted.

The diagnosis of CM is confirmed by the demonstration of pathologic mast cell infiltration of the skin. SM requires involvement of at least one extracutaneous tissue by clonal mast cells (bone marrow is the most commonly involved organ). Diagnostic criteria for CM, SM, and variant presentations of SM are summarized in Table 18-17.

Course and prognosis

Life expectancy can be quite variable, ranging from only a few months in aggressive SM variants to nearly normal life spans in more indolent disease. CM in children tends to have an indolent course and often is associated with spontaneous regression. Adults with CM rarely may evolve to SM. The presence of cutaneous involvement in SM appears to confer an indolent behavior, whereas lack of skin involvement is associated with aggressive behavior. Predictive factors of poor prognosis in SM include older age at onset of symptoms, absence of CM, low platelets, hypoalbuminemia, hepatosplenomegaly, anemia, and elevated LDH.

Management

Treatment of CM includes H1 and H2 antihistamines, cromolyn and other mast cell stabilizers, topical or intralesional glucocorticoids, and psoralen and ultraviolet A

phototherapy. Adults with chronic CM may require long-term continuous or intermittent symptomatic treatment.

For adult patients with indolent variants of SM, treatment of mediator-related symptoms with combinations of H1 and H2 antihistamines, leukotriene antagonists, proton pump inhibitors, cromolyn, and other mast cell stabilizers may be sufficient to alleviate symptoms. Patients with SM should carry epinephrine in an injectable form available at all times for managing anaphylaxis. Aspirin and nonsteroidal anti-inflammatory drugs have been helpful for some patients with flushing and syncope, but hypersensitivity to these drugs is relatively common and must be excluded. Accordingly, a major goal in the management of mastocytosis is the avoidance of known triggers, which can include opioid analgesics, such as morphine and codeine, which are known mast cell degranulators, as well as anesthesia, stress, and infection. Off-label use of IFN α can be helpful for patients with painful skeletal lesions or mast cell tumors that threaten bony integrity. Those with osteoporosis can be treated with calcium and/or bisphosphonate therapy when indicated. Patients with severe or refractory mediator-related symptoms can be considered for cytoreductive therapy.

The aggressive variants of SM may progress to end-stage organ fibrosis or failure and may be complicated by pathologic fractures, severe cytopenias, or both. Patients with evidence of end-organ damage without major bony complications may benefit from off-label use of IFN α with or without corticosteroids (in cases with incipient end-organ damage), although most responses are only partial. More rapid cytoreduction is seen with single-agent cladribine, given at $5\text{ mg/m}^2/\text{d}$ or 0.13 to 0.17 mg/kg/d as a 5-day treatment cycle every 4 to 6 weeks, which has induced clinical and laboratory responses (ie., decreased serum tryptase and urinary histamine metabolites) in patients with symptomatic SM. Patients receiving cladribine require close follow-up for supportive care due to the myelosuppressive effects of the treatment.

The crucial role of KIT in normal mast cell development and the evidence that *KIT* mutations may be important in

SM pathogenesis prompted treatment of mastocytosis patients with Tyrosine kinase inhibitors (TKIs). Because of its inhibitory properties against KIT, imatinib was the first to enter the clinical arena and has regulatory approval in patients who lack the *KIT* D816V mutation. The presence of *KIT* D816V mutation confers resistance to imatinib by affecting the catalytic pocket of the *KIT* protein, preventing imatinib from binding and exerting its inhibitory activity. Therefore, *KIT* mutation analysis is important in therapeutic decision making in SM. A trial of imatinib should be considered for those with aggressive SM who lack the D816V mutation or whose *KIT* mutation status is unknown.

Midostaurin is a multikinase inhibitor that has displayed potent activity against both wild-type and mutant *KIT*. On this basis, an open-label study of midostaurin 100 mg twice a day in continuous 28-day cycles until progression or intolerable toxicity for patients with aggressive SM was conducted. The overall response rate was 60%, including a major response rate of 45% and partial response rate of 15%. Major response was defined as normalization of ≥ 1 SM-related organ damage findings (C findings) such as albumin levels, resolution of liver transaminitis and other liver function tests, relief of ascites and pleural effusion, improvement of hemoglobin and platelet levels, and reversal of weight loss. The median duration of response was not reached in patients with ASM or MCL, and it was 12.7 months for patients with SM-AHN. The median overall survival was 44.4 months in responders and 15.4 months for nonresponders (28.7 months for all patients). The median change in mast cell burden, as measured by reduction in tryptase, was 57%. The most clinically relevant treatment-emergent toxicities were nausea, vomiting, and diarrhea as well as myelosuppression, especially in patients with preexisting cytopenias. Based on the results of this study, midostaurin was granted regulatory approval for the treatment of advanced SM.

Patients who fail to respond to cladribine or midostaurin, and those who progress to mast cell leukemia, should enroll in a clinical trial or can be considered for multiagent antileukemic chemotherapy. Allogeneic HCT should be considered for younger patients with aggressive SM who achieve a remission with chemotherapy. In a retrospective analysis of 57 patients, 28% achieved complete remission (CR) after transplant; for all subtypes, the overall survival (OS) was 57% at 3 years, including 74% for SM-AHN, 43% with ASM, and 17% for mast cell leukemia. The diagnosis of MCL (vs others) and reduced-intensity conditioning regimens (vs myeloablative) were associated with an inferior overall survival.

Symptomatic SM in the presence of a non-mast cell hematologic neoplasm should be treated as indicated both for the hematologic malignancy and for the SM complications. Generally, the underlying non-SM malignancy determines the overall clinical course, although in cases in which aggressive forms of SM coexist with low-grade myeloid neoplasms, the aggressive SM may take precedence.

Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2*

The WHO recognizes three rare conditions classified as myeloid/lymphoid neoplasms associated with marked and persistent eosinophilia and chromosomal rearrangements, leading to constitutive activation of the *PDGFRA*/*PDGFRB*, *FGFR1*, or *PCM1-JAK2* genes (Table 18-18).

These are separate entities from CEL and from HES, which are subcategories of MPNs. Although the partner gene involved heavily influences the clinical features, separate consideration needs to be given to *PDGFRA*- and *PDGFRB*-rearranged eosinophilic disorders because they carry major therapeutic relevance due to the exquisite sensitivity to imatinib therapy.

Table 18-18 Molecular genetic abnormalities in myeloid/lymphoid neoplasms associated with eosinophilia

Disease	Presentation	Genetics	Treatment
<i>PDGFRA</i>	Eosinophilia ↑ Serum tryptase ↑ Marrow mast cells	Cryptic deletion at 4q12 <i>FIP1L1-PDGFRA</i> , at least seven other partners	Respond to TKI
<i>PDGFRB</i>	Eosinophilia Monocytosis mimicking CMML	t(5;12)(q32;p13.2) <i>ETV6-PDGFRB</i> , at least 30 other partners	Respond to TKI
<i>FGFR1</i>	Eosinophilia Often presents with T-ALL or AML	Translocations of 8p11.2 <i>FGFR1</i> -various partners	Poor prognosis; do not respond to TKI
<i>PCM1-JAK2</i>	Eosinophilia Rarely presents with T-ALL or B-ALL Bone marrow shows left-shifted erythroid predominance and lymphoid aggregates	t(8;9)(p22;p24.1) <i>PCM1-JAK2</i>	May respond to JAK2 inhibitors

Adapted from Arber DA et al, *Blood*. 2016;127(20):2391–2405.

Myeloid/lymphoid neoplasms with *PDGFRA* or *PDGFRB* rearrangements

CLINICAL CASE

A 52-year-old mechanic suffered a stroke of unclear etiology, followed by recurrent headache, rhinorrhea, wheezing, weight loss of 15 lb (~7 kg), diarrhea, night sweats, pruritus, and lower-extremity edema. He underwent a routine blood test, including a CBC, which showed the following: WBCs = $15 \times 10^9/L$, Hgb = 10.3 g/dL, MCV = 89 fL, and platelets = $224 \times 10^9/L$. The most notable feature on the WBC differential was an eosinophilia with an AEC of $2.7 \times 10^9/L$. There were no circulating blasts in the peripheral blood. Workup for an underlying connective tissue disease, other neoplastic process, and parasitic infection was negative. A CT scan of the sinus revealed thickening of the right sphenoid sinus. Total IgE was elevated (IgE = 283 KU/L). CT of the chest showed patchy opacities consistent with bronchiolitis or vasculitis. CT scan of the abdomen and pelvis confirmed splenomegaly. Transthoracic echocardiography showed a diminished ejection fraction of 30% and the presence of restrictive cardiomyopathy. FISH for the *CHIC2* deletion, a surrogate for the *FIP1L1-PDGFR*A fusion, was positive in 56% of cells.

Although the true incidence of *PDGFRA*-related neoplasms is not known, it is clear these are rare hematologic disorders. These neoplasms are considerably more common in men than in women (male-to-female ratio, 9:1 to 17:1) and usually are diagnosed between the ages of 25 and 55 years (median age of onset is late 40s). Approximately 5% to 10% of patients in industrial countries who present with idiopathic hypereosinophilia can be found to have the *FIP1L1-PDGFR*A fusion. Similarly, *PDGFRB*-related neoplasms are extremely uncommon disorders, and the true incidence of it is not completely known. In fact, among >56,000 cytogenetically defined cases from the Mayo Clinic, only 0.04% exhibited the t(5;12) translocation. In another prospective study, of 556 cases with MPN, only 10 with *PDGFRB* rearrangements were noted. *PDGFRB*-related neoplasms are more common in men than in women (male-to-female ratio, 2:1), with a median age of onset in late 40s.

Pathobiology

PDGFRA and *PDGFRB* are members of the class III receptor tyrosine kinase family, which also includes *KIT* and *FLT3*. The pathobiology of these molecular lesions are described in the “Driver mutations” section of this chapter.

Clinical features and diagnosis

PDGFRA- and *PDGFRB*-related neoplasms are multisystem disorders associated with bone marrow and peripheral blood eosinophilia. The most common presenting signs and

symptoms are weakness, fatigue, cardiopulmonary symptoms, myalgias, rash, and fever. Splenomegaly is a common finding, with a minority of patients also presenting with hepatomegaly. Organ damage occurs as a result of release of cytokines or direct organ infiltration by eosinophils and possibly mast cells. The most serious complication of *PDGFRA*- and *PDGFRB*-related neoplasms is cardiac in nature, including endomyocardial fibrosis with ensuing restrictive cardiomyopathy.

The most prominent diagnostic feature of patients with *PDGFRA*-related neoplasms is the presence of peripheral blood mature eosinophilia. An elevated serum tryptase is usually also present. Bone marrow biopsy demonstrates marked hypercellularity with increased mature and precursor eosinophils. It is important to note that *FIP1L1-PDGFR*A rearrangements are not exclusively associated with an MPN phenotype, because these rearrangements are also associated with presentations of acute leukemia. Fibrosis can be present. Immunophenotyping is typical for activated eosinophils with expression of CD23, CD25, and CD69. The gold standard for the diagnosis of these neoplasms is demonstration of the fusion gene. As mentioned, most cases of CEL-NOS present with normal karyotype; thus, FISH and Reverse transcription polymerase chain reaction (RT-PCR) are preferred methods of testing. FISH testing relies on the probe for the *CHIC2* gene, which is deleted uniformly in patients with the *FIP1L1-PDGFR*A fusion gene. RT-PCR can be used in cases with a high clinical suspicion and negative FISH testing. RT-PCR is also used for monitoring of disease response and for minimal residual disease testing.

Patients with *PDGFRB*-related neoplasms tend to present with anemia and thrombocytopenia, along with leukocytosis neutrophilia or monocytosis; features characteristic of CMML including a monocytic leukocytosis with associated eosinophilia are often seen. Confirmation of diagnosis for *PDGFRB*-related neoplasms requires demonstration of MPN with prominent eosinophilia and occasional neutrophilia or monocytosis and the presence of the *ETV6-PDGFRB* fusion gene or an alternative *PDGFRB* gene rearrangement. The classic t(5;12)(q31-q33;p13) can be detected easily by conventional metaphase analysis, so FISH or RT-PCR usually is used for the confirmation of diagnosis and determination of the fusion gene. The bone marrow is hypercellular, with increased fibrosis, and mast cells can be increased in number.

Course and prognosis

In the pre-imatinib era, the prognosis of patients with *PDGFRA*- or *PDGFRB*-related neoplasia was poor; the median survival did not exceed 1 to 2 years. Patients generally had advanced disease, with congestive heart failure ac-

counting for 65% of the identified causes of death. However, imatinib has positively altered the natural history. An observed 5-year survival rate of 80%, decreasing to 42% at 15 years, was noted in one retrospective study. In another series reported from the Mayo Clinic, with long-term follow-up, the median survival was not reached, and 18 of 22 (82%) were alive, though 2 leukemic transformations were reported.

Management

The mainstay of therapy for patients with *PDGFRA*- and *PDGFRB*-related neoplasms is the use of imatinib. One of the earliest pivotal reports identifying *FIPL1-PDGFRα* as a therapeutic target of imatinib was reported by Cools et al in 2003. Following this report, investigators from the National Institute of Health (NIH) reported on improved hematological parameters, including reversal of bone marrow fibrosis, along with molecular remissions in five of six patients. Subsequently, several single- and multi-institution studies have looked at the efficacy of low to conventional doses of imatinib for the treatment of *PDGFRA*-related neoplasms. These studies report remarkably similar results, where patients found to have *PDGFRA* gene rearrangements have rapid, deep, and durable responses to low to conventional doses of imatinib (100 to 400 mg/d). Along these lines, the European Leukemia Net (ELN) reported the results of 11 patients treated for at least 12 months with imatinib. Overall, 11 of 11 evaluable patients achieved at least a 3-log reduction in *FIPL1-PDGFRα* fusion transcripts, and 9 of 11 patients achieved a complete molecular remission. Similarly, an Italian multicenter study demonstrated high levels of durable (median, 25 months) and complete molecular remissions in 27 patients with *PDGFRA*-related neoplasms. In those with known eosinophilic heart disease, steroids are recommended concurrently with imatinib during the first 1 to 2 weeks of therapy given prior reports of cardiogenic shock.

Interestingly, in a retrospective report of 44 patients by the French Eosinophil Network, complete hematologic and molecular remission was obtained in 44 of 44 and 43 of 44, respectively. Among 11 patients in whom imatinib was discontinued, 5 remained in remission (range, 9 to 88 months). However, this strategy requires confirmation in a prospective setting, and indefinite therapy is recommended. Compared with *BCR-ABL1*-positive CML, kinase domain mutations that confer resistance to imatinib therapy including T674I and D842V are rare in *FIPL1-PDGFRα* rearrangement-positive disease. Other tyrosine kinase inhibitors have been used in this setting with only modest and transient benefit.

In 2002, Apperley et al reported four patients with *PDGFRB*-related neoplasms treated with imatinib 400 mg

daily, and normalization of blood counts occurred within 4 weeks, the t(5;12) translocation was undetectable by 12 to 36 weeks, and transcript levels decreased in those with the *ETV6-PDGFRB* fusion. A report on 12 patients with *PDGFRB*-related neoplasms who received imatinib therapy for a median of 47 months revealed normalization of peripheral blood cell counts and disappearance of eosinophilia in 11; 10 had complete resolution of cytogenetic abnormalities and decrease or disappearance of fusion transcripts as measured by RT-PCR. A retrospective report of an expanded cohort of 26 patients with a median follow-up of 10.2 years (imatinib duration, 6.6 years) reported a 90% 10-year survival, a 96% response rate, and that no patient with complete cytogenetic (N=13) or molecular (N=8) response lost their response.

Myeloid/lymphoid neoplasms with *FGFR1* rearrangement

This uncommon and heterogeneous group of neoplasms arise from pluripotent hematopoietic stem cells and are associated with rearrangements in the *FGFR1* gene and eosinophilia. Formerly known as 8p11 myeloproliferative syndrome or 8p11 stem cell syndrome, *FGFR1*-related neoplasms can present as classic MPNs, precursor B- or T-cell lymphoblastic leukemia, or AML. *FGFR1*-related neoplasms have been reported across a wide age range (3 to 84 years), and the median age of diagnosis is 32 years. Females constitute approximately 40% of the cases. It is important to note that eosinophilia is not always present despite the name of the diagnostic category.

Pathobiology

The molecular consequences of *FGFR1* rearrangements are remarkably well described for such an uncommon neoplasm. The pathobiology of this disorder is described in the “Driver mutations” section of this chapter.

Clinical features and diagnosis

Clinical manifestations include fever, weight loss, and night sweats. Lymphadenopathy is common in patients with lymphomatous presentation. Hypercatabolism and splenomegaly are common features of AML and MPN patients. Diagnostic criteria outlined in the 2016 WHO classification include the presence of an MPN with prominent eosinophilia and occasional neutrophilia or monocytosis or the presence of AML or precursor B- or T-cell lymphoblastic leukemia and the presence of *FGFR1* rearrangement. The most common chromosomal translocation associated with *FGFR1*-related neoplasms is t(8;13) (p11;q12), which results in expression of the ZNF198-*FGFR1* fusion TK. Fifteen fusion gene partners have been described in *FGFR1* rearrangement neoplasms,

Clinical features	Skin lesions: urticaria pigmentosa and Darier sign Constitutional and mediator release symptoms Anaphylaxis Osteopenia, fracture Hepatosplenomegaly				
Diagnostic criteria	Major criterion: Multifocal, dense infiltrates of mast cells (≥ 15 mast cells in aggregates) in bone marrow and/or extracutaneous organ(s) Minor criteria: a) $>25\%$ with atypia, immaturity, or spindle-shaped appearance b) Mast cell expression of CD117 with CD2 and/or CD25 c) <i>KIT</i> D816B d) Serum tryptase persistently >20 ng/mL				
Subtypes	Indolent No AHN No "C" findings	Advanced systemic mastocytosis			
	Aggressive SM No MCL ≥ 1 "C" finding (malabsorption, skeletal lesions, impaired liver function, splenomegaly, or cytopenias)	SM-AHN SM in addition to WHO criteria for AML, MDS, MPN, or lymphoma	Mast cell leukemia $\geq 10\%$ neoplastic mast cells in blood or $\geq 20\%$ on aspirate		
Clinical features	Trigger avoidance H1/H2 blockers, proton pump inhibitors, leukotriene antagonists, cromolyn, IFN α Clinical trial, midostaurin, cladribine, chemotherapy Allogeneic SCT				

Figure 18-8 Systemic mastocytosis is diagnosed in the presence of one major criterion and one minor criterion or in the presence of at least three minor criteria. See Table 18-17.

Figure 18-9 The hypereosinophilias discussed in this chapter are typically characterized by sustained eosinophilia and end-organ consequences, the most severe of which can be cardiac in nature. HES is distinguished by absence of a clonal marker, and steroids are considered a frontline therapeutic option. It is critical to recognize *PDGFRA/PDGFRB*-rearranged neoplasms given their remarkable sensitivity to low-dose imatinib. See text for diagnostic criteria (Table 18-5).

Clinical and laboratory features	Cardiac, pulmonary, neurological, and dermatological involvement Splenomegaly Lymphadenopathy (<i>FGFR1</i>) Sustained eosinophilia ($>1.5 \times 10^9/L$) Anemia, thrombocytopenia Bone marrow fibrosis				
Subtypes	HES	<i>PDGFRA/PDGFRB</i> and <i>FGFR1</i> -rearranged neoplasms or with <i>PCM1-JAK2</i>			
Additional diagnostic features	Persistent, primary eosinophilia with end-organ damage; no increased blood/bone marrow blasts; no clonal disease or aberrant T-cell population	<i>PDGFRA</i>	<i>PDGFRB</i> May have features in common with CMML, JMML, MDS/MPN-U, atypical CML	<i>FGFR1</i>	<i>PCM1-JAK2</i>
First-line treatment options	Steroids	Imatinib (initiate steroids if elevated cardiac troponin) and/or cardiac dysfunction	Clinical trial or induction chemotherapy followed by allogeneic transplantation	JAK2 inhibitor (ruxolitinib) followed by allogeneic transplantation	HU, IFN α , corticosteroids (if organ damage is present) empiric trial of imatinib, allogeneic transplantation

including *CEP110*, *FGFR1OP1*, *FGFR1OP2*, *TRIM 24*, *MYO18A*, *HERVK*, and *BCR*.

Course and prognosis

The prognosis for patients with *FGFR1*-related neoplasms is very poor, with evolution to AML typically occurring within 1 to 2 years. The clinical aggressiveness and diminished awareness about the features of this entity and the lack of approved therapies make the management of these patients very challenging.

Management

Early intensive chemotherapy followed by allogeneic SCT remains the only potential curative therapy for patients with *FGFR1*-related neoplasms. Interestingly, midostaurin has demonstrated *in vitro* activity against one subtype of the *FGFR1* fusion gene and, in one patient, resulted in improved leukocytosis, splenomegaly, and lymphadenopathy and 6 months of clinical stability prior to transplantation. Additional TKIs with anti-*FGFR1* activity are being evaluated, including a selective *FGFR1* inhibitor ([ClinicalTrials.gov](#): identifier NCT03011372).

Myeloid/lymphoid neoplasms with *PCM1-JAK2* rearrangement

A patient with t(8;9)(p22;p24) was described by Stewart et al in 1990, and the identification of the *PCM1-JAK2* fusion gene by Reiter et al followed in 2005. Across the more than 30 cases reported in the literature, there is a marked male predominance. The median age at the time of diagnosis is approximately 50 years. In addition to eosinophilia, hepatosplenomegaly is a common clinical feature. However, it is important to note that eosinophilia is not present in all cases. The bone marrow is often left shifted with erythroid predominance and lymphoid aggregates. Many patients also are found to have myelofibrosis. It can also rarely present as acute T- or B-cell lymphoblastic leukemia. Myeloid/lymphoid neoplasms with t(8;9)(p22;p24.1);*PCM1-JAK2* was added to the 2016 WHO diagnostic classification schema as a provisional entity.

Pathobiology

Several breakpoint locations affecting *JAK2* and *PCM1* have been identified in patients with t(8;9) that lead to a fusion product with the coiled-coil domains of *PCM1* and the tyrosine-kinase domain of *JAK2*. The oligomerization of *PCM1-JAK2* results in constitutive activation of *JAK2*.

Management

The prognosis for patients with *PCM1-JAK2*-related neoplasms is very poor, with evolution to AML typically

occurring within 1 to 2 years. Two case reports have highlighted complete hematologic remissions and cytogenetic responses in patients with *PCM1-JAK2* treated with ruxolitinib. However, the duration of these remissions can be variable, and allogenic transplantation should be considered irrespective of response to ruxolitinib.

KEY POINTS

See also Figures 18-8 and 18-9.

- CNL is characterized by a sustained mature, neutrophilic leukocytosis, often with splenomegaly, and *CSF3R* mutations identified in substantial proportions of patients. CNL is a progressive MPN, with HU and allogeneic SCT as treatment options, though TKI may have a future role depending on the type of *CSF3R* mutation that is present.
- Mastocytosis includes cutaneous and systemic mastocytosis; the latter is more commonly identified in adults and includes both indolent and aggressive subtypes. Aggressive mastocytosis can be associated with an underlying hematological malignancy or can manifest as mast cell leukemia. Consequences stem from mediator release or organ infiltration, and most patients have *KIT D816V* mutations. Treatments are supportive, including H1/H2 blockade and mast cell stabilizers. Along with bisphosphonates, IFN is an option for patients with severe refractory bone involvement. Patients whose disease lack the *KIT 816V* mutations are sensitive to imatinib, where midostaurin is an active agent independent of *KIT* mutation status.
- Myeloproliferative neoplasms with eosinophilia include CEL and those with *PDGFRA/PDGFRB*, *FGFR1*, and *PCM1-JAK2* rearrangements. Cardiac involvement can be a source of morbidity and mortality, especially in those with CEL and *PDGFRA*-rearranged disease. Characteristic findings include sustained eosinophilia, often with monocytosis in those with *PDGFRB*-rearranged disease. Those with *FGFR1* rearrangements may also have splenomegaly and lymphadenopathy. Patients with *PDGFRA/PDGFRB*-rearranged disease are uniquely sensitive to imatinib, which has had a very positive impact on prognosis for these rare neoplasms.

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Acquired marrow failure syndromes: aplastic anemia, paroxysmal nocturnal hemoglobinuria, and myelodysplastic syndromes

AMY E. DEZERN AND CATHERINE SMITH

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The online version of this chapter contains educational multimedia components on pathogenesis and treatment of PNH and on CHIP, ICUS, CCUS, and MDS and the role of the clonal evolution.

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Introduction

The bone marrow failure (BMF) syndromes comprise a rare and heterogeneous group of clinically and pathologically distinct disorders associated with cytopenias and failure of normal hematopoiesis. In BMF disorders, the inability of hematopoiesis to meet physiological demands for the production of healthy blood cells can result in either pancytopenia or cytopenias involving specific lineages (eg., anemia, thrombocytopenia, neutropenia). The etiology of marrow failure in an individual patient can be multifactorial or related to a single cause. These various disorders may either be extrinsic or intrinsic to the marrow. An example of an extrinsic cause is the inappropriate immune response that results in aplastic anemia, whereas the hematopoietic progenitor or stem cell defects that underlie the myelodysplastic syndromes (MDS) are intrinsic. BMF syndromes can be acquired or, more rarely, congenital.

The range of molecular mechanisms responsible for congenital marrow failure states is broad, including abnormal DNA damage response (Fanconi anemia [FA]), defective ribogenesis (Diamond-Blackfan anemia [DBA]), abnormal telomere dynamics (dyskeratosis congenita [DC]), and altered hematopoietic growth factor receptor/kinase signaling (congenital amegakaryocytic thrombocytopenia). Similar mechanisms may underlie some acquired marrow failure syndromes, such as acquired haploinsufficiency for ribosomal protein RPS14 in MDS associated with chromosome 5q deletion, which parallels heterozygous ribosomal protein mutations observed in DBA.

This chapter focuses on *acquired* marrow failure syndromes, including aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), and MDS. There is also discussion of other more recently realized issues with idiopathic cytopenia of undetermined significance (ICUS), clonal hematopoiesis of indeterminant potential (CHIP), and clonal cytopenia of undetermined significance (CCUS). For discussion of inherited marrow failure syndromes such as Fanconi anemia, dyskeratosis congenita, and Diamond-Blackfan anemia, please refer to Chapter 16.

Aplastic anemia

Definition

Idiopathic AA is a hematopoietic stem cell disorder associated with reduced bone marrow cellularity and decreased hematopoiesis. This decreased hematopoiesis

may disproportionately affect one or two cell line lineages in early stages of the disease, but AA is ultimately associated with trilineage hypoplasia. Classification and prognosis in AA are related to the depth of cytopenias in the peripheral blood. Severity drives the therapeutic decisions. Severe AA (SAA) is defined by depression of blood counts involving at least two hematopoietic lineages (ie., absolute reticulocyte count $<60 \times 10^9/L$, absolute neutrophil count $<0.5 \times 10^9/L$, or platelet count $<20 \times 10^9/L$) and bone marrow hypocellularity (<30%, excluding lymphocytes). Very severe AA has an absolute neutrophil count of $<0.2 \times 10^9/L$, whereas moderate AA is characterized by depression of blood counts not fulfilling the definition of severe disease (Table 19-1).

Classification

AA may be acquired and idiopathic, or it can arise in the context of an inherited marrow failure syndrome. This distinction carries profound implications for management and treatment. For example, immunosuppression is a therapeutic option in acquired AA, whereas this treatment modality is ineffective in inherited forms of marrow failure. AA is a diagnosis of exclusion, and systemic causes for pancytopenia should be ruled out. The diagnosis of AA usually is reserved for naturally occurring conditions and excludes those patients with a history of cytotoxic chemotherapy or exposure to ionizing radiation.

Epidemiology

AA is primarily a disease of children and younger adults. Another peak in incidence rate occurs in patients 60 years of age and older, although, in these older patients, some reported cases of AA may actually represent hypoplastic MDS. AA is rare in Western Europe and the United States (less than 2 cases per million in the population per year) and more common in Asia, with an incidence rate of 3.9 cases per million per year in Bangkok, 6 cases per million per year in rural areas of Thailand, and 14 cases per

million per year in Japan. This increase in Asian AA patients compared to white or mixed AA patient has been attributed a genetic disposition (Asian human leukocyte antigen[HLA] type and nucleotide polymorphisms) rather than environmental factors. Both males and females are equally affected. AA can be acquired or constitutional. Idiopathic acquired AA is perceived as a T-cell-mediated autoimmune process with the immune attack at the level of the CD34-positive hematopoietic stem cell. Idiopathic AA is more common than AA associated with toxins, pregnancy, or hepatitis. The association of AA with drug exposure has been of great interest for decades. However, the level of evidence linking AA to specific drugs is variable. The nonsteroidal anti-inflammatory agents indomethacin, diclofenac, and butazones (such as phenylbutazone), anti-thyroid medication (propylthiouracil), certain anticonvulsants (such as hydantoins, carbamazepine), and certain antibiotics such as chloramphenicol and gold salts are more clearly associated with development of AA. Environmental exposure to benzene is also linked to marrow failure in the literature.

Hepatitis-associated AA accounts for 2% to 5% of cases of AA in Europe and 4% to 10% of cases in East Asia. AA has been reported to occur in 28% to 33% of patients requiring ortho-topic liver transplantation for fulminant non-A, non-B, and non-C hepatitis. This seronegative hepatitis in patients with posthepatitis AA does not appear to be caused by any of the currently known hepatitis viruses and often is referred to as hepatitis/AA syndrome. An immune pathogenesis following a putative trigger is suspected, but the precise mechanism is unknown. AA evolves with a typical delay of several weeks to months after the episode of hepatitis, usually after the transaminitis has peaked and begins to trend down.

Etiology and pathogenesis

Regardless of the etiology, the hallmark of AA is the reduction in hematopoiesis, as reflected by marrow

Table 19-1 Classification of aplastic anemia by severity

Peripheral blood cytopenias	Nonsevere (moderate) aplastic anemia (not meeting criteria for severe disease)	Severe aplastic anemia (any two of three)	Very severe aplastic anemia (meets criteria for severe disease and absolute neutrophils <200)
Bone marrow cellularity	<25%	<25%	<25%
Absolute neutrophil count		$<0.5 \times 10^9/L$	$<0.2 \times 10^9/L$
Platelet count		$<20,000/\mu L$	
Reticulocyte count		$<1.0\%$ corrected or $<60,000/\mu L$	

*Very severe aplastic anemia is reserved for patients who fulfill criteria for SAA but with an absolute neutrophil count of $<0.2 \times 10^9/L$.

histology, low numbers of marrow CD34 cells, diminished numbers of long-term culture-initiating cells (a surrogate measure of hematopoietic stem cells [HSCs]), and poor hematopoietic colony formation in cells obtained from an aplastic marrow. Clinical response to immunosuppressive therapy (IST) targeting T cells (eg, antithymocyte globulin [ATG]), described further below in the “Immunosuppressive therapy” section, supports an immune-mediated pathogenesis of AA. AA is thought to be initiated by recognition and destruction of HSCs by cytotoxic T lymphocytes, which recognize some unknown antigen present on HSCs via their HLA class I molecule. (Figure 19-1A). There is ample data to support this hypothesis, including the presence of T cells at diagnosis that decrease or disappear with IST. Additionally, there is further evidence of increases in proinflammatory cytokines, including interferon γ and tumor necrosis factor α (TNF α), from aberrantly activated immune cells and stromal microenvironments that also contribute to BMF in AA. This has been attributed to Fas apoptosis signal (FAS)-mediated apoptosis. Although diverse triggers, such as viruses or chemical hazards, may serve as inciting events in individual cases, the final autoimmune pathway appears to be uniform. It is this pathogenesis and applied IST that may allow for future clonal evolution discussed below.

Clinical presentation

The resultant cytopenias in a patient with a diagnosis of AA cause the symptoms. At presentation, the clinicians should consider the workup shown in Table 19-2. Patients

typically have fatigue, weakness, pallor, and headaches due to anemia. Often, patients have petechiae of the skin and mucous membranes, epistaxis, and gum bleeding related to severe thrombocytopenia. More severe hemorrhage in the central nervous system or gastrointestinal tract would be atypical at the time of diagnosis. Fever and infections can also be seen in these patients as a consequence of a compromised immune system. Acquired AA patients who are identified early due to abnormalities in routine laboratory testing may have no physical manifestations of their disease. AA most often arises in a previously healthy patient who has no history of malignancy and no exposure to cytotoxic drugs or history of radiation exposure. A family history of marrow failure or dysmorphology may help identify inherited causes of pancytopenia, such as FA or DC. Drug and chemical exposures should be queried in the interview, but these are notoriously difficult to evaluate quantitatively as the history is subject to recall bias. Confirmation of a causal relationship is difficult to ascertain in practice, and management is not likely to differ from those cases without a putative trigger. Discontinuation of a drug strongly suspected to be associated with the onset of pancytopenia is reasonable for a few weeks; however, a prolonged observation period of several weeks to months before initiation of therapy is not recommended, especially when pancytopenia remains severe.

Splenomegaly and hepatomegaly are not typical features of AA and should point toward another diagnosis. Short stature, musculoskeletal abnormalities (particularly radial ray anomalies), dysplastic nails, skin rashes, oral leukoplakia, exo-

Figure 19-1 Immune-mediated pathogenesis of AA. (A) AA is thought to be initiated by recognition and destruction of HSCs by CTLs, which recognize some unknown antigen present on HSCs via their HLA class I molecule. (B) During and/or after immune-mediated BM destruction, a rapid expansion of residual cells (which escaped destruction) occurs, whereby cells carrying mutations achieve clonal dominance and may progress to malignant proliferation. CTL, cytotoxic T cell. BM, bone marrow. Redrawn from Ogawa S. *Blood*. 2016;128(3):337–347.

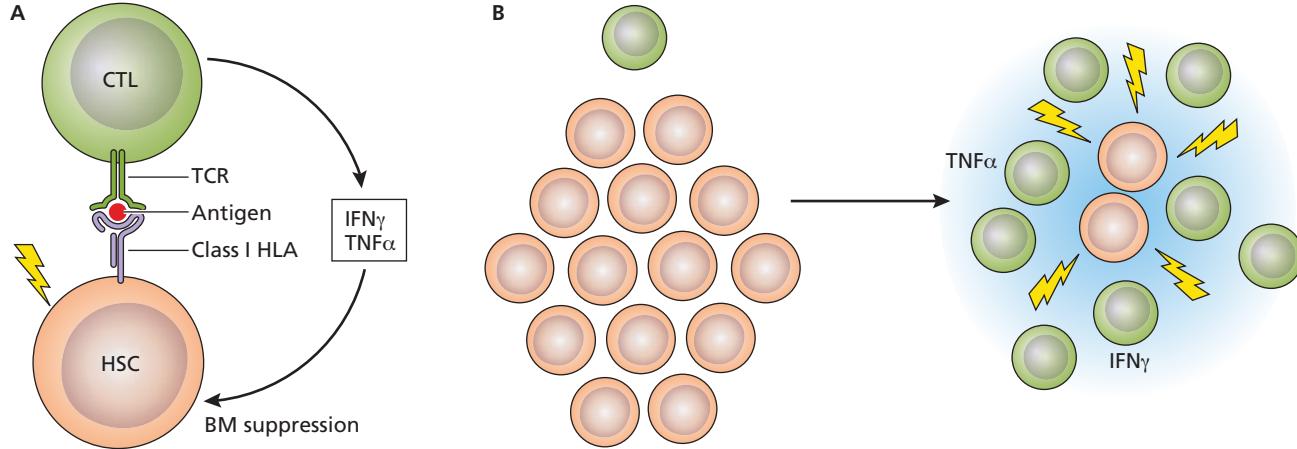


Table 19-2 Initial evaluation for presumed aplastic anemia

Patient history	Duration of cytopenias (are previous blood counts known?) Medications (prescribed and over-the-counter supplements) Exposures Transfusions Immunodeficiencies or autoimmunity
Family history	Constitutional abnormalities Malignancies Other family members with cytopenias
Physical examination	Height (in context of mean parental height) Limb abnormalities Skin and nail abnormalities (café au lait spots, nail dystrophy, pale patches)
Laboratory	Peripheral blood Complete blood count with differential Reticulocyte counts Chemistries Transaminases and bilirubin Hepatitis serologies Beta-HCG (consider even if intercourse is not explicitly stated) FLAER flow cytometry assay Chromosomal breakage tests (diepoxybutane or mitomycin C) Telomere length and mutational analysis (if DC suspected) Bone marrow Aspirate and biopsy Flow cytometry (including quantitative CD34) Cytogenetics FISH Consideration of gene panel

HCG, human chorionic gonadotropin.

crine pancreatic insufficiency, or other congenital anomalies may suggest an inherited BMF state (see Chapter 16). The absence of characteristic physical findings or a suggestive family history does not rule out an inherited marrow failure syndrome, which can manifest in adulthood with apparent acquired AA or MDS and no physical stigmata. The detection of genetic defects associated with FA or DC in some adults with AA but without dysmorphology has blurred the distinction between inherited and acquired forms of marrow failure. It is important to investigate past medical history carefully about earlier blood count abnormalities, macrocytosis, or relevant pulmonary (fibrosis) or liver disease (cirrhosis) as well as review the patient's family while keeping familial or inherited syndromes in the differential diagnosis.

The peripheral blood in AA shows pancytopenia usually with a relative lymphocytosis, but is otherwise unremarkable. The bone marrow biopsy in these patients is characterized by hypocellularity. The criteria for the diagnosis of AA (Table 19-1) require either bone marrow with <25% of the normal cellularity or bone marrow with <50% normal cellularity in which less than 30% of the cells are hematopoietic, as the bone marrow in AA can occasionally have increased lymphocytes, which are predominantly mature T cells. In patients with abundant lymphoid infiltrates, immunohistochemical or flow cytometric evaluation may be warranted to rule out an underlying lymphoma. The bone marrow aspirates in AA are correspondingly paucicellular, and the few hematopoietic elements seen do not show overt dysplastic changes. However, erythroid dysplasia alone can be seen in AA and is not diagnostic of MDS. The myeloid cells may show a left shift, but blasts are not increased. Flow cytometric evaluation of the bone marrow in AA is characterized by a relative lymphocytosis. CD34-positive blasts are rare, and the few seen will show no phenotypic abnormality. If the blasts are phenotypically abnormal or increased, then a diagnosis of hypoplastic MDS should be entertained. Patients with AA may also have small PNH clones; these clones are detected using specialized flow cytometric techniques, as discussed in detail in the PNH section. The identification of a PNH clone may be helpful diagnostically, as PNH clones are not present in inherited or in acquired causes of BMF in younger patients; however, as small PNH clones can also be seen in MDS, this method cannot be used to solely differentiate PNH from MDS in older patients. The presence or absence of a PNH clone is also important to document in AA as its presence may predict a good response to IST. AA is associated with normal cytogenetics. An abnormal karyotype in a patient with a hypocellular bone marrow suggests a diagnosis of hypocellular MDS, although some investigators believe that certain chromosomal abnormalities, such as trisomy 8 or deletion 13q, can still be consistent with an AA diagnosis and not a marker of clonality to define MDS.

Lastly, acquired AA has been associated with telomere length changes. Approximately one-third of patients with acquired AA have short telomeres at the time of initial presentation. In fact, 10% of patients with acquired AA have mutations in *TERT* (the telomerase gene) or *TERC* (the telomerase RNA template gene), both of which lead to short telomeres. Although the use of telomere length as a treatment response biomarker is still not standard, short telomere length may be predictive of a higher relapse rate and could be a risk factor for clonal evolution.

Table 19-3 Differential diagnosis of pancytopenia with a hypocellular bone marrow

Acquired aplastic anemia
Inherited aplastic anemia
Fanconi anemia
Dyskeratosis congenita
Shwachman-Diamond syndrome
Amegakaryocytic thrombocytopenia
Reticular dysgenesis
Hypoplastic myelodysplastic syndromes
Large granular lymphocytic leukemia (rare)
Hypoplastic paroxysmal nocturnal hemoglobinuria (PNH/ aplastic anemia)

Differential diagnosis

When evaluating a patient with pancytopenia and a hypocellular marrow, the physician must exclude a number of other conditions before a diagnosis of AA can be made (Table 19-3; Figure 19-2). The most common disorders include MDS, acute leukemia, PNH, or an inherited syndrome. Also in the differential diagnosis are myelofibrosis, hairy cell leukemia, certain infections (tuberculosis, HIV), nutritional deficiency (eg., anorexia nervosa), or T-cell large granular lymphocyte (T-LGL) disease (T-LGL populations can coexist with AA or MDS). The diagnostic approach to the patient with pancytopenia (Table 19-2) includes the following: history including medications, previous chemotherapy or radiation exposure, occupational toxic exposures, HIV risk factors, family history; physical examination, paying particular attention to presence of organomegaly, lymphadenopathy, or congenital abnormalities (short stature, nail dystrophy, abnormalities in skin, arms, head, eyes, mucosa, or skeletal); complete blood count, including reticulocyte count and peripheral smear examination; liver function tests, vitamin B₁₂ and folate levels, lactate dehydrogenase (LDH), haptoglobin, and flow cytometry for PNH evaluation; bone marrow aspirate and biopsy with cytogenetic studies; and chromosome fragility tests, particularly patients less than 40 years of age for Fanconi anemia (FA) testing.

The presence of dysplastic immature hematopoietic cells or blast cells should lead to a diagnosis of hypoplastic MDS or acute leukemia. Similarly, marrow cytogenetic analysis may detect a cytogenetic abnormality diagnostic of lymphoid or myeloid leukemic disorders. Hairy cell leukemia frequently presents as pancytopenia with difficulty in aspirating the marrow, or a “dry tap,” along with splenomegaly. Pancytopenia can arise in the setting of anorexia nervosa as an epiphénoménon of the eating disorder, pos-

sibly because of multiple micronutrient deficiencies. Pancytopenia in this setting is associated with a hypocellular marrow with serous fat atrophy. Vitamin B₁₂ and folate levels should be determined in all patients, although the marrow in vitamin B₁₂ or folate deficiency is typically hypercellular and megaloblastic rather than hypocellular. HIV infection or AIDS is associated with cytopenia, morphologic dysplasia, and marrow hypocellularity in ~10% of cases. A careful inquiry into HIV risk factors and an HIV test are prudent.

T-LGL is a rare condition characterized by circulating T-cells bearing the CD57 marker of effector or cytotoxic T-cells. T-LGL, like PNH, can coexist with AA or MDS. T-LGL disease should be considered if increased LGLs are noted on the peripheral blood smear or if the patient has concomitant systemic autoimmune disease such as rheumatoid arthritis, which is known to be associated with T-LGL. Single-lineage cytopenia is more common in T-LGL with clinical presentation of isolated neutropenia most typical or an anemia. Flow cytometry and testing for a clonal T-cell receptor gene rearrangement is appropriate when T-LGL is suspected. These patients also have a higher prevalence of *STAT3* mutations.

Another possible underlying cause of AA is FA, which can present with cytopenias in younger patients without other classic features of the disease. Therefore, diepoxybutane or mitomycin C testing to exclude chromosome fragility is important in patients with newly diagnosed AA <40 years of age, even in the absence of musculoskeletal abnormalities. FA is discussed further in Chapter 16 on inherited diseases of marrow failure.

The distinction between AA and hypoplastic MDS may be difficult to make, and increasing evidence suggests that immune-mediated mechanisms similar to those postulated to cause AA may contribute to the cytopenias associated with some cases of hypoplastic MDS and also normocellular or hypercellular MDS, even in the absence of a preceding diagnosis of AA. Such evidence includes the identification of clonal-activated cytotoxic T-cell populations in both AA and MDS, the coexistence of PNH and T-LGL clones in both AA and MDS, and improved blood counts in a subset of MDS patients treated with IST (see next section on myelodysplastic syndromes). Hypolobated neutrophils, dysplastic megakaryocytes, or abnormally localized and increased immature precursors favor a diagnosis of hypoplastic MDS rather than AA. Sometimes the only way to make the distinction between AA and MDS is by detection of an abnormal cytogenetic clonal population, but even this may not be diagnostic of MDS because some cytogenetically abnormal clones can be observed transiently in AA.

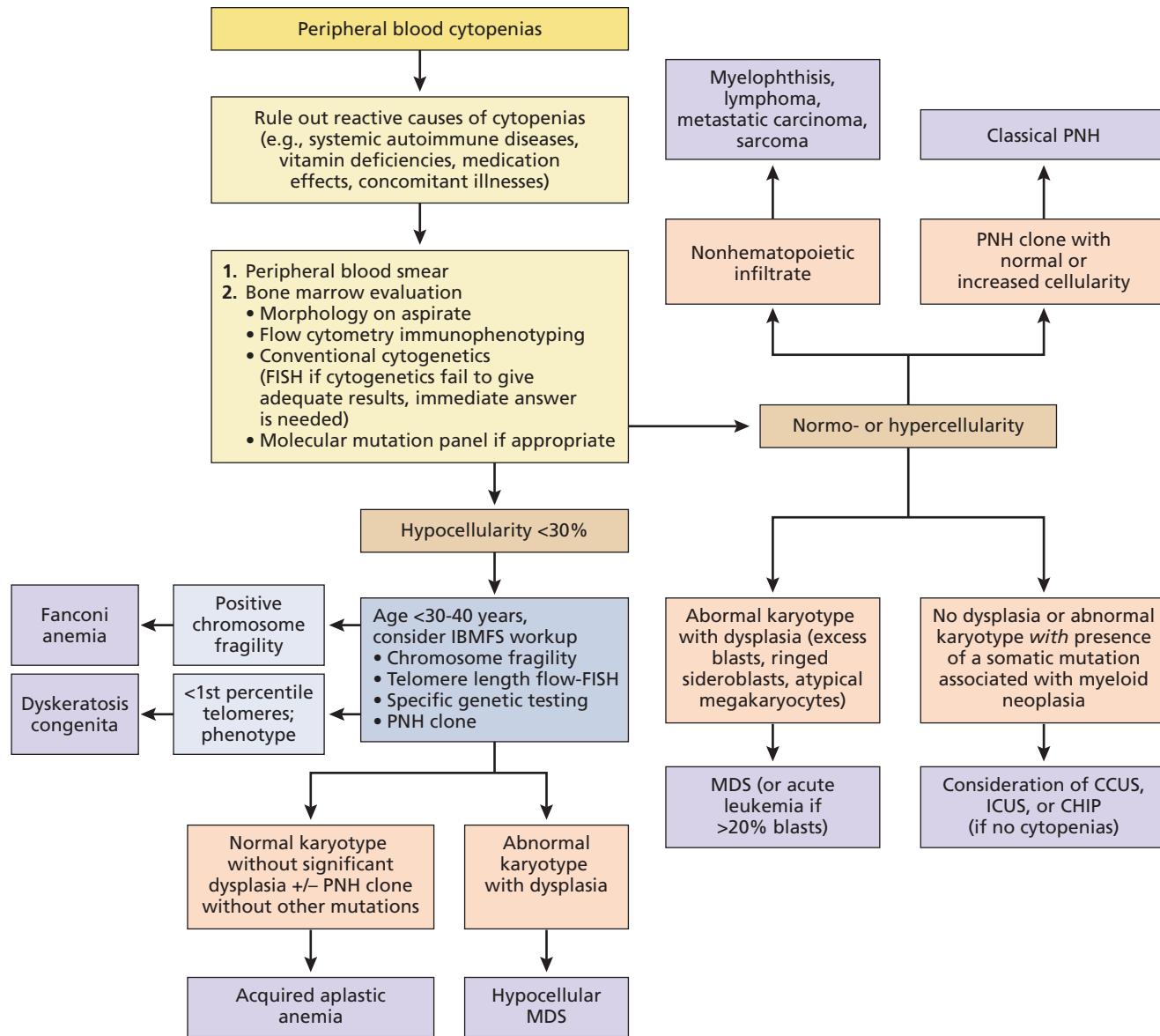


Figure 19-2 Diagnostic algorithm of primary marrow causes of pancytopenia. In patients with a hypocellular marrow, the main differential is between hypocellular MDS and AA. Normal cytogenetics and no significant dysplastic changes favor AA, whereas more pronounced dysplasia (micromegakaryocytes, left shift myelopoiesis with increase in blasts, significant dyserythropoiesis) and an abnormal cytogenetics favor MDS. Patients with AA and a PNH clone are classified as AA/PNH, which is distinct from classical PNH. It is important in appropriate patients to consider inherited causes of the marrow failure (IBMFS). In those with normal or increased marrow cellularity, differential includes a nonhematopoietic marrow infiltrating process (lymphomas, metastatic carcinoma, or sarcomas), MDS, and other primary marrow disorders (including AML if >20% blasts). More recently as next-generation sequencing panels are sent for molecular mutations, the diagnoses of CHIP, CCUS, and ICUS are being made as well.

KEY POINTS

- AA is a diagnosis of exclusion and can result from intrinsic stem cell defects, immunologic impairment of hematopoiesis, or toxic effect of an exogenous exposure.
- PNH clones are frequently seen in patients with AA.

- Chromosome abnormalities favor an MDS diagnosis over AA, but both karyotypic changes and clonal somatic mutations can sometimes be seen in patients with AA.
- Chromosome fragility tests are important to exclude FA in children and younger adults <40 years of age presenting with idiopathic marrow failure.

Therapy

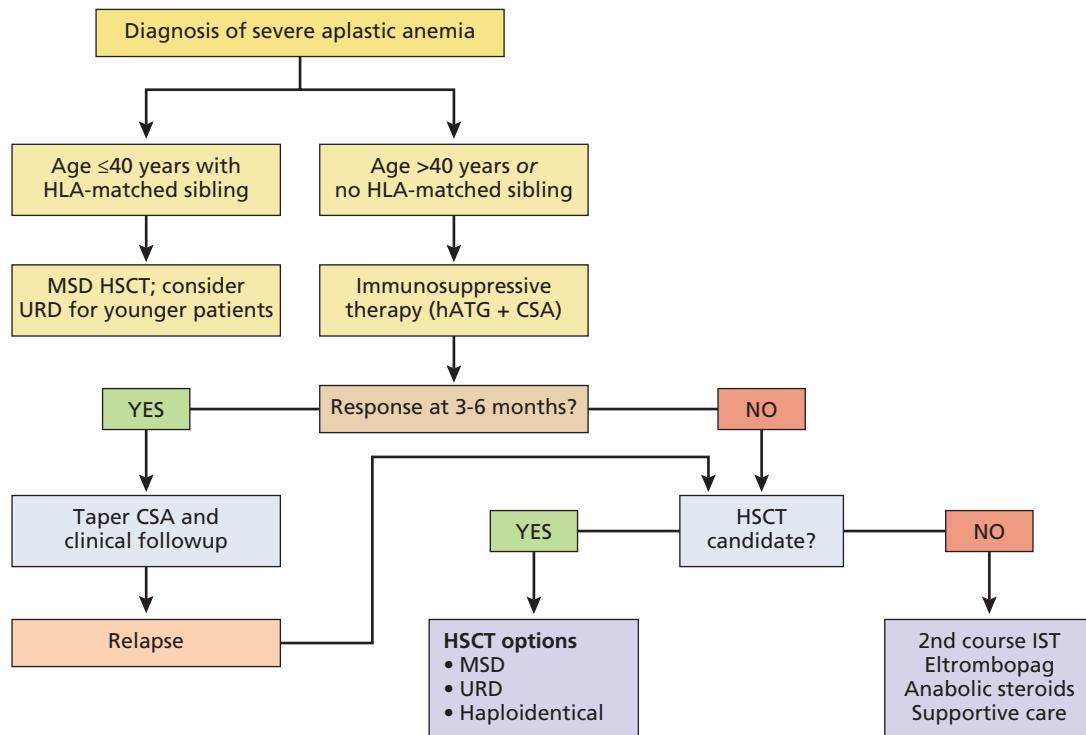
Without treatment, almost all patients with SAA or very severe AA eventually will succumb to infection or to hemorrhagic complications. Therefore, such patients require urgent therapy once a diagnosis is confirmed. The decision to treat patients with AA is based on disease severity. Definitive treatment with either IST or allogeneic hematopoietic stem cell transplantation (HSCT) is necessary for patients with SAA (Figure 19-3). The standard of care for nonsevere AA is not established. Except for cases in which there is transfusion dependence, therapy is optional because survival is not affected by treatment. Rarely, patients with moderate AA can spontaneously recover normal hematopoiesis. Spontaneous remission is most often seen with drug-induced AA and usually occurs within 1 to 2 months of discontinuing the offending drug. Once the severity criteria are fulfilled, the type of treatment recommended is influenced by the patient's age and the availability of a matched sibling donor (MSD). A younger age (typically

<40 years of age) and the presence of an MSD favor the use of allogeneic HSCT, while older age (>40 years old) and absence of an MSD favor the use of IST, which typically uses a combination of ATG and cyclosporine (CsA). Attention to the timeline for reconstitution of hematopoiesis in these patients is critical for good outcomes.

Supportive care, transfusions, and hematopoietic growth factors

Supportive care is instituted to sustain blood counts (both hemoglobin and platelets) and alleviate symptoms and risks associated with pancytopenia and consists of transfusion of irradiated, leukocyte-depleted blood products (blood and/or platelets) due to the risk for alloimmunization from chronic transfusions. Transfusions from related potential donors should be avoided because doing so could increase the risk of subsequent graft rejection. If the patient is cytomegalovirus negative, it is best to use cytomegalovirus-negative blood products or leukocyte-depleted products. The role

Figure 19-3 Algorithm for initial management of SAA. In patients who are not candidates for an upfront MSD HSCT, high dose immunosuppression (likely with h-ATG plus CSA and consideration of eltrombopag) should be the initial therapy. Response assessment occurs at 3 to 6 months, and the decisions on further intervention for nonresponders is based again on severity of disease. In patients who have persistent neutrophil count $<0.2 \times 10^9/L$, use of salvage therapies earlier is prudent. HSCT is favored to reconstitute hematopoiesis in appropriate patients. In those who are not suitable for transplantation and a repeat course of immunosuppression due to advanced age, comorbidities, lack of donor, poor performance, or personal preference, non-HSCT options can be considered earlier after refractoriness to initial course of therapy.



of preventive antibiotics in neutropenic patients is not well defined.

Fungal and bacterial infections are a major cause of death in patients with SAA. However, an active fungal infection should not delay more definitive therapy, such as IST or HSCT. There is no standardized approach to antibiotic therapy in AA at any age group. Vigilance as well as proactive use of prophylactic antibiotics (when deemed clinically appropriate), antivirals, and antifungals are recommended. Where possible, it is prudent to avoid agents associated with high rates of bone marrow suppression.

Granulocyte colony stimulating factor or granulocyte-macrophage colony stimulating factor and erythropoiesis stimulating agents have a limited role in AA. Most patients with AA have an elevated serum erythropoietin level and do not respond to recombinant erythropoietin. Although typical AA will not respond to myeloid growth factors either (ie, granulocyte colony-stimulating factor [G-CSF] or granulocyte-macrophage colony-stimulating factor), some patients do improve neutrophil counts, and these growth factors may have a role in decreasing infectious morbidity while awaiting definitive treatment with immunosuppression or HSCT. In several randomized trials, the addition of G-CSF to standard ATG and cyclosporine therapy did not improve the rates of hematologic response rate or survival. More recently, the thrombopoietin receptor agonist eltrombopag was studied in combination with ATG/CSA and results discussed below in section on immunosuppressive therapy.

Corticosteroids are ineffective, increase the risk of infection, and should not be used as therapy in AA. The role of corticosteroids in SAA is limited to serum sickness prophylaxis with concurrent ATG administration. Androgens may have a supportive role in some patients throughout the treatment course of AA. Androgens, however, should not be used as primary upfront therapy.

KEY POINTS

- If transfusions are needed in a patient with AA, use irradiated, leukocyte-depleted blood products.
- Transfusions should not be from family members (especially in transplant candidates).
- Transfusions should be used judiciously but should not be withheld in symptomatic anemic transfusions should be used judiciously or in those at higher risk for bleeding.
- AA does not usually respond to G-CSF or erythropoietin.
- Corticosteroids should not be used as therapy in AA except as prevention of serum sickness in patients receiving ATG.

Hematopoietic stem cell transplantation

Adolescents and young adults (age <40 years) meeting the criteria for severe disease who have an HLA-MSD should proceed directly to HSCT, as this is potentially curative. An advantage of HCT over standard IST is a marked reduction in the risk of relapse and abrogation of the risk for the development of clonal disorders such as MDS and PNH. Despite this, the risks of acute and chronic graft-versus-host disease (GVHD) remain a challenge after HSCT when donors other than matched siblings are used.

In AA, the pretransplantation conditioning regimen primarily is administered to provide immunosuppression, which enables the donor stem cells to engraft and also eliminate activated immune cells that may be causing the marrow aplasia. Cyclophosphamide (50 mg/kg/d × 4 days) with or without ATG is commonly used for conditioning before stem cell transplantation. Although this regimen is nonmyeloablative, the immunosuppression is sufficient to allow engraftment in most cases. Avoidance of total body irradiation and busulfan reduces transplant-related complications such as mucositis, GVHD, second malignancies, and infertility. Alternative regimens using fludarabine, cyclophosphamide, and antithymocyte globulin are increasingly being used. Survival rates following matched sibling allogeneic bone marrow transplantation (BMT) have steadily improved since the 1970s largely because of improved supportive care, improved typing, and better GVHD prophylaxis. Bone marrow has been a traditional source for the stem cell graft, but the use of peripheral blood stem cells has gained in popularity in the past 10 to 15 years. This practice has resulted in an untoward consequence in transplanted AA patients, where several reports in the recent years from Europe and the United States show an increase rate of GVHD, with stem cells derived from mobilized peripheral blood when compared with a bone marrow source. In contrast to allogeneic HSCT undertaken for malignant disorders, where GVHD offers potential graft-versus-tumor benefits, GVHD is to be avoided at all costs in the AA setting, because its occurrence is associated with decreased survival and long-term quality of life. Thus, bone marrow is the preferred source of HSCs in AA patients undergoing HSCT.

Late BMT-related complications such as chronic GVHD occur in up to one-third of patients, with many of these patients requiring long-term therapy for their GVHD. Standard prophylactic therapy for GVHD includes a calcineurin inhibitor (cyclosporine or tacrolimus) and methotrexate or post-transplant cyclophosphamide. Patient age and the type of allograft (HLA-matched sibling, unrelated, or mismatched donors) are the most important factors influencing

outcome. In patients under 30 years of age, the cure rate after HLA-matched sibling BMT ranges from 70% to 90%. However, the risk of GVHD steadily increases with age, leading to reduced survival. A recent Cochran review concluded that no firm conclusions can be drawn about the comparative effectiveness of first-line allogeneic HSCT of HLA-matched sibling donors and first-line IST of patients with acquired SAA.

For older patients, reduced-intensity transplantation conditioning regimens using low doses of total-body irradiation or fludarabine have shown promise in reducing rejection rates. In recent years, however, outcomes with matched unrelated-donor HSCT have improved likely because of more stringent donor selection with high-resolution-molecular tissue typing, less toxic and more effective conditioning regimens, and higher quality transfusion and antimicrobial supportive care. In some reports in children, outcomes with a matched-unrelated HSCT have compared favorably to those observed with sibling donors, and this treatment modality is becoming the preferred salvage treatment modality in younger patients who fail an initial course of immunosuppression when a matched-unrelated histocompatible donor is available.

Outcomes with mismatched-unrelated umbilical cord donors are not as favorable, with higher rates of graft rejection, infectious complications, acute and chronic GVHD, and transplant-related mortality. Newer results in haploidential HSCT have increasing success with lower toxicity than previously reported. These alternative donor transplants usually are undertaken at the time that refractory or relapsed disease is diagnosed (Figure 19-3). There are ongoing investigations into utilization of alternative donors earlier in a patient's course.

KEY POINTS

- Outcomes with HSCT are better in younger patients (especially patients <20 years old); in patients >40 years old, transplantation-related mortality and morbidity may be increased somewhat.
- Bone marrow is the preferred source of stem cells in AA, not peripheral blood stem cells, unlike the situation with hematological neoplasms.
- Alternative transplantation should be reserved for patients for whom an initial course of immunosuppression has failed.

Immunosuppressive therapy

The principal immunosuppressive agent used in SAA is ATG, which is manufactured by delivering human T cells

to a horse or rabbit. The immunized animal then produces antibodies against antigens expressed on the surface of a T cell, which subsequently are harvested and purified. The resulting polyclonal animal serum has lymphocytotoxic properties, and administration to humans leads to varying degrees of lymphocyte depletion. Several *in vitro* and *in vivo* differences are observed between the two types of ATG despite a similar manufacturing process. Rabbit ATG (r-ATG) has a longer half-life and results in a more durable lymphocyte depletion compared to horse ATG (h-ATG). A difference in T-cell binding affinity, cytokine release, and T-cell subset depletion and reconstitution has also been shown to be distinct between the ATGs.

Initial investigations using h-ATG or CsA alone in AA were succeeded by studies of h-ATG and CsA in combination, with improved response rates over monotherapy, becoming the standard regimen. Multiple efforts to add further immunosuppression to improve outcomes beyond h-ATG/CsA have been disappointing. Addition of mycophenolate mofetil, G-CSF, or sirolimus did not improve hematologic responses or decrease the relapse and clonal evolution rates. The use of more lymphocytotoxic agents such as r-ATG, alemtuzumab, or cyclophosphamide led to worse outcomes than with h-ATG/CsA in randomized studies, due to a lower response rate and/or excess toxicities.

Recently the thrombopoietin receptor agonist eltrombopag was studied in a larger 92-patient prospective trial at the National Institutes of Health in combination with ATG/CsA. This trial demonstrated higher rates of response compared to historical controls (80% to 94% compared to 66%), and response was associated with a longer duration of eltrombopag exposure (up to 6 months). The addition of eltrombopag was well tolerated, with only rash as a severe adverse event in two patients. With early tapering of the CSA in the initial phases of the study, there was a relapse rate of 32%, resulting in an amendment to continue the CSA for 2 years. Concerns of increased clonal evolution have been postulated with clonal cytogenetic evolution in seven patients at 2 years, but this has not been shown to be more than previous reports, and longer-term follow-up is ongoing. Many now consider the addition of eltrombopag to IST as standard of care for initial treatment of SAA.

The usual time to response to h-ATG/CsA therapy in SAA is approximately 10 to 12 weeks. In most studies, responses are defined as achieving blood counts that no longer fulfill criteria for severe disease, as well as transfusion independence. Total restoration of blood counts will occur in a minority of patients, and recovery can be protracted.

The overall response rate at 3 months in patients receiving h-ATG/CsA is between 60% and 80%. Hepatitis-

associated and drug-related AA appears to be as equally responsive to IST as idiopathic AA. Although most patients who respond to IST do so by 6 months, in a small minority of patients, time to recovery may be longer. Achieving hematologic response (partial or complete) to immunosuppression is very important in SAA because it strongly associates with long-term survival.

Both horse- and rabbit-derived ATGs have activity in SAA, with most of the experience with horse occurring in the upfront setting and experience with rabbit in the salvage setting. A repeat course of r-ATG and CsA may be given to h-ATG-refractory patients, which results in additional responses in approximately 35% of patients. In responders to h-ATG/CsA, relapse has been reported in 35% of patients by 5 years. Relapses can be related temporally to the discontinuation of CsA or to the reduction of its dose. Cyclosporine should be continued for at least 6 months. The benefit of a taper in abrogating or reducing relapse rates has not been confirmed in prospective studies; however, most practicing hematologists institute a slow CsA taper after 6 months in an attempt to prevent hematologic relapses. Relapsed patients may respond to an increased dose or reintroduction of CsA or a second course of r-ATG, which results in hematologic responses in about 60% to 70% of patients. Approximately 25% of patients remain chronically dependent on CsA to maintain adequate blood counts. Aggressive taper is usually not beneficial to the patient, whereas active titration to avoid side effects (especially nephrotoxicity) is prudent.

The greater lymphocytotoxicity of r-ATG and its effectiveness in salvaging refractory and relapsed SAA patients prompted its use as initial therapy with the anticipation that it would be superior to h-ATG. In a randomized study, however, results with r-ATG were disappointing. The hematologic response rate with r-ATG was 37% compared with 68% for horse ATG at 6 months, and survival was inferior in the r-ATG arm. These results suggest that h-ATG/CsA remains the preferred first-line IST in SAA. As alternative therapy to h-ATG/CsA, high-dose cyclophosphamide has been used, with response rates comparable to that of h-ATG/CsA but with early reports suggesting fewer rates of relapse and clonal evolution.

The thrombopoietin receptor agonist eltrombopag approach has demonstrated a role in refractory AA, as a small trial showed improvements in blood counts in patients with SAA who were refractory to at least one course of immunosuppression. A hematologic response rate of approximately 40% has been reported with this single agent, with multilineage responses observed. This outpatient oral therapy was well tolerated and is approved for use after insufficient response to initial IST with ATG and CSA alone.

Long-term follow-up and prognosis

Clonal outgrowth with secondary hematological malignancies and impaired fertility are among the most worrisome late effects of IST and HSCT. Patients treated with IST have a 1% to 5% chance of secondary hematological malignancies with clonal evolution to MDS or clinical PNH. Routine monitoring (generally annual) should be performed.

Although 40% to 50% of patients with AA will have PNH clones at presentation, most are small, and evolution to frank PNH is relatively infrequent. PNH that occurs after treatment, however, frequently is subclinical and rarely is associated with overt hemolysis or thrombosis. More concerning is evolution to MDS, which most frequently is associated with either monosomy 7 or a trisomy 8 karyotype. Evolution to MDS can occur in up to 15% to 20% of patients in the first 20 years after diagnosis, an event usually associated with a decrease in blood counts or refractoriness to immunosuppression. The prognosis of patients with chromosome 7 abnormalities is generally poor, whereas those with trisomy 8 can respond to IST.

Other cytogenetic abnormalities can be identified in follow-up of AA, which may not necessarily signify progression to MDS. Some of these abnormalities may be transient and may not be associated with dysplastic marrow findings, worsening in blood counts, or refractoriness to further therapies. The exception is the appearance of monosomy 7, which commonly is associated with frank dysplasia, with the only curative approach being an HSCT from a related or alternative donor.

Molecular testing is an evolving area of active research in the AA field and is primarily still performed on a research rather than a clinical basis. As many as 60% to 70% of acquired AA patients demonstrate clonality at the time of diagnosis, using sensitive next-generation sequencing and array-based karyotyping (comparative genomic hybridization) modalities. Unfortunately, these clones are often not eliminated post therapy and are frequently the source of relapse and/or progression. Recurrent genetic abnormalities in *ASXL1*, *DNMT3A*, *TET2*, and *BCOR* genes have been recently described in AA but their relevance is not clearly defined, and clones bearing these markers may disappear with time. Discussion of this clonal hematopoiesis are seen below in the “Clonal hematopoiesis” section and their evolution depicted in Figure 19-1B.

Rarely, AA may develop in pregnancy. Spontaneous remission can occur in 25% to 30% of patients, often upon birth or termination of the pregnancy. CsA may be a safe drug antenatally in such patients. Complications appear to be more likely in pregnant patients with low platelet counts and associated PNH.

Overall survival is approximately 70% in patients over 16 years of age. HSCT using an MSD is indicated as a front-line approach in children and patients up to 20 years. However, approximately 70% of patients do not have an MSD. Further, clonal evolution often occurs in patients with AA and these patients do less well long term.

KEY POINTS

- Allogeneic stem cell transplantation from a matched sibling donor is the treatment of choice for patients with SAA in children and young adults.
- For older patients, those without sibling donors, and those who refuse transplantation or have significant comorbidities that preclude HSCT, immunosuppression with h-ATG plus cyclosporine combination should be initiated as soon as possible once the diagnostic workup is completed.
- In patients without matched sibling donors, regardless of age, h-ATG plus cyclosporine should be the preferred initial treatment. H-ATG is superior to r-ATG as a first-line therapy.
- Outcomes with matched unrelated-donor transplantation have been improving and may be considered as the preferred salvage treatment in children and young adults who fail an initial course of immunosuppression and have a histocompatible unrelated donor.
- The combination of ATG and cyclosporine is more effective than single-agent immunosuppression in SAA.
- Relapses occur in about one-third of responders to h-ATG plus cyclosporine but often respond well to reinstitution of IST.
- Repeat courses of r-ATG and cyclosporine may be given to refractory patients, resulting in a salvage rate of approximately 35%.
- Eltrombopag is approved for therapeutic use in SAA patients who have an insufficient response to initial IST and is an option for those who are not eligible for HSCT due to lack of a histocompatible donor, age comorbidities, or personal preference.
- Clonal evolution to MDS can occur in 10% to 20% of patients long term.
- Higher-risk transplant modalities from mismatched-unrelated, haploidentical, or umbilical cord donors should be reserved for patients refractory to IST or relapsed or performed on clinical trials.

Paroxysmal nocturnal hemoglobinuria

In acquired AA, PNH clones can be detected by flow cytometry in 40% to 50% of cases, but these are usually small (<10% of cells). A PNH clone can expand later in the course of disease leading to frank hemolysis; this occurs most commonly in patients with larger preexisting PNH clones at diagnosis. PNH clones can remain stable over time

or reduce in size, having no clinical consequence. Indicators of the presence of a PNH clone include elevated LDH, absent haptoglobin, increased reticulocytes, and erythroid predominance in the marrow.

Definition

PNH is a rare clonal HSC disorder that manifests with a chronic intravascular hemolytic anemia from uncontrolled complement activation, a propensity for thrombosis, and BMF. The hemolysis is largely mediated by the alternative pathway of complement. These clinical manifestations result from the lack of specific cell surface proteins, CD55 and CD59, on PNH cells resulting from a somatic mutation in the *PIGA* gene in HSCs, which results in failure to synthesize the glycosylphosphatidylinositol (GPI) anchor.

Pathophysiology

Hemolysis in PNH is complement mediated and is a direct result of the mutated PNH cells acquiring a deficiency of complement regulatory proteins. In PNH, because of the defect of the enzyme encoded by the mutant *PIGA* gene (Figure 19-4A), the first step in biosynthesis of the GPI anchor protein (AP) cannot be completed normally (Figure 19-4B), and all GPI-anchored proteins are absent on the surface of progeny cells of all hematopoietic lineages derived from the affected stem cell with increased susceptibility to hemolysis (Figure 19-4C and D).

The intravascular hemolysis in PNH is due to the lack of GPI-anchored proteins (CD55 and CD59) that attenuate complement activation on the surface of erythrocytes. Depending on the type of mutation in the *PIGA* gene, various degrees of CD55 and CD59 deficiency can occur. Patients with PNH may have in their circulation an admixture of normal complement-resistant red blood cells (so-called PNH I cells), as well as mildly (PNH II) or markedly (PNH III) abnormal complement-sensitive cells. The difference in the proportion of these red blood cell populations contributes to the variability in intravascular hemolysis seen in patients.

PNH patients have a propensity for thrombosis. Several theories have been postulated to account for this hypercoagulability, but the mechanism has not been clearly defined. It is believed that thrombophilia in PNH is related to the degree of hemolysis and thereby indirectly related to the size of PNH clone. Possible prothrombotic pathways include platelet activation by complement components, procoagulable microparticles derived from GPI-deficient erythrocytes, or slowing of the microcirculation because of vasoconstriction induced by products of hemolysis. It also has been suggested that intravascular hemolysis exposes red blood cell phospholipids that may serve to initiate coagulation.

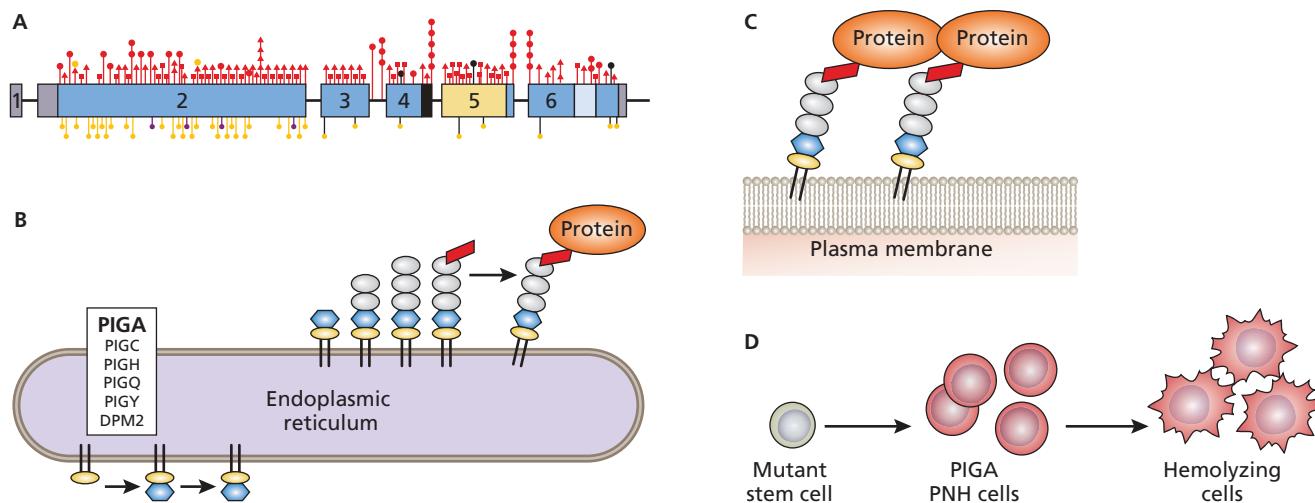


Figure 19-4 Pathogenesis of PNH. (A) In hematopoietic stem cells, acquired somatic mutations of the *PIGA* gene may occur. This controls the key step in the biosynthesis of GPI anchor proteins. (B) GPI anchor biosynthesis takes place in the endoplasmic reticulum. *PIGA* is one of seven subunits involved in the first step of GPI anchor biosynthesis. (C) After multiple steps including protein attachment to the GPI anchor and fatty acid remodeling, the GPI anchored protein should be transported to the plasma membrane. This cannot occur in PNH patients. (D) These mutations (in panel A) can decrease the function or totally inactivate the enzyme encoded by *PIGA*. As a consequence, all proteins using this type of anchor are deficient from the membrane of affected progeny derived from the mutant stem cells and cause the PNH phenotype and hemolysis.

PNH is also a disorder of marrow failure. PNH clones expand only in the context of immune-mediated BMF, explaining the close association between AA and PNH. According to the most predominant hypothesis, PNH stem cells, which can be found in very low frequencies in healthy individuals, have a selective advantage in certain circumstances of immune dysregulation. Under conditions of T-cell-mediated immune attack on HSCs, GPI-deficient stem cells appear to thrive due to selective survival advantage

compared with healthy stem cells, which facilitate their expansion. This close association between immune-mediated depletion of normal stem and progenitor cells explains the coexistence of hematopoietic failure and frequent cytopenias related to impaired blood cell production (Figure 19-5).

Laboratory findings and diagnosis

The diagnosis of PNH is both a laboratory and a clinical diagnosis, which can show numerous and varied presentations.

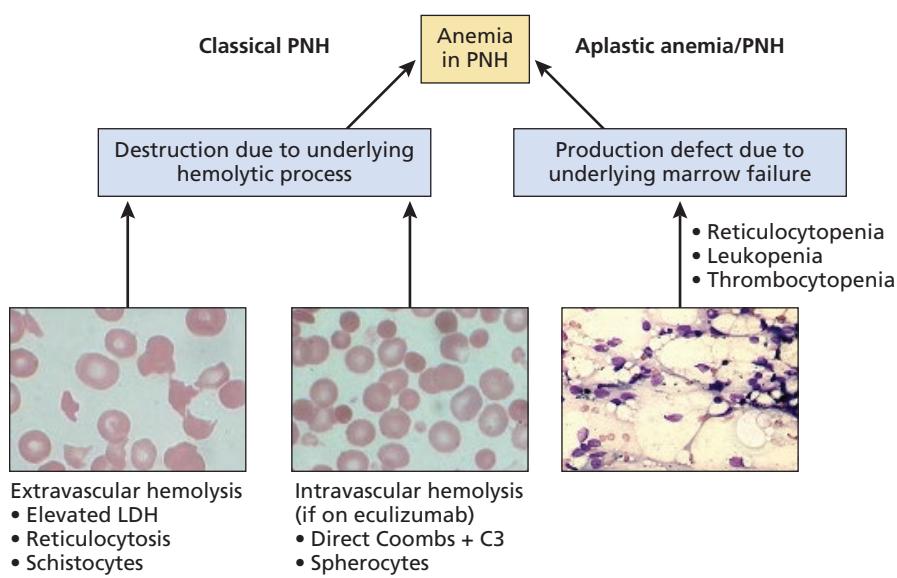


Figure 19-5 Mechanisms of anemia in PNH.

Anemia in PNH can be a result of increased RBC destruction due to intravascular hemolysis of GPI-deficient RBCs, decreased production of RBCs due to immune-mediated BMF, or a combination of these two mechanisms. Hemolysis can be compensated for by increased production (patients with increased reticulocytes) or compensation may be inadequate (patients with low reticulocyte counts).

It may present with a Coombs-negative hemolytic anemia, pancytopenia, abdominal pain, renal impairment, hemoglobinuria, and/or thrombosis. PNH can arise *de novo* or evolve from acquired aplastic anemia. The laboratory diagnosis of PNH formerly relied on the demonstration of abnormally complement-sensitive erythrocyte populations such as the Ham test or sucrose lysis test. These two tests are primarily of historical interest. Currently, the diagnosis of PNH is secured by abnormal laboratory measures including a reticulocyte count, lactate dehydrogenase levels, complete blood count indicative of hemolysis, and peripheral blood flow cytometry to detect the deficiency of the GPI-AP. This absence of GPI-APs is detected after staining cells with monoclonal antibodies (eg, CD55, CD59) and/or a reagent known as fluorescein-tagged proaerolysin variant (FLAER) that binds a portion of the GPI anchor. The erythrocytes may be classified as type I, II, or III PNH cells, as noted above. It should be noted that testing of a PNH clone solely in erythrocytes is not adequate for evaluation of PNH, because hemolysis and transfusions may greatly underestimate the size of the clone. For these reasons, granulocyte and monocyte clones are frequently detected when erythrocyte clones are not. Hematopathologists have recently published guidelines for diagnosis of PNH using flow cytometry. In patients with brisk hemolysis associated with PNH, macrocytic anemia due to compensatory reticulocytosis typically is present (if hematopoiesis is not suppressed), but some PNH patients with iron deficiency due to chronic urinary iron losses may have microcytic red blood cell indices. Elevated LDH and absent haptoglobin together with urine hemosiderin indicate the presence of intravascular hemolysis. Patients with PNH who do not receive transfusions develop various degrees of iron deficiency anemia over time. Various degrees of thrombocytopenia and neutropenia also may be present in patients with PNH associated with AA. In the absence of AA, the bone marrow shows relative expansion of erythroid series, and most often is hypercellular.

There is no universally accepted classification scheme. Recently the PNH International Registry classified PNH into the following three categories: (1) hemolytic or classical PNH; (2) AA-PNH, and (3) intermediate PNH. Patients with hemolytic PNH tend to have near-normal neutrophil and platelet counts, an LDH >2 times the upper limit of normal, a normocellular bone marrow, an elevated reticulocyte count, and a relatively large population of PNH granulocytes (usually >50%). AA-PNH patients are more deeply pancytopenic and tend to have a hypocellular bone marrow, a relatively low reticulocyte count, and a smaller percentage of PNH granulocytes. It is also important to recognize that these categories have limi-

tations and a patient's classification can change over time. For example, patients with AA-PNH may experience improved hematopoiesis associated with expansion of their PNH clone and later meet criteria for hemolytic PNH. Less commonly, patients with hemolytic PNH may develop AA-PNH.

KEY POINTS

- PNH is an acquired clonal HSC disorder characterized by deficiency of GPI-linked proteins in blood and bone marrow cells due to a somatic mutation in the *PIGA* gene.
- Patients with PNH experience chronic hemolytic anemia (intravascular) from uncontrolled complement activation. They may also suffer from a propensity for thrombosis and BMF (indicated by leukopenia and/or thrombocytopenia in addition to anemia).
- Flow cytometric techniques to identify cell populations lacking GPI-linked proteins, such as CD55 and CD59, confirm the diagnosis of PNH and are used to estimate the size of PNH clone.

Clinical manifestations

Chronic hemolytic anemia of various degrees is the most common manifestation of PNH. Despite the name of the disease, hemoglobinuria with darker-stained urine at a particular time of the day is reported by only a minority of patients. Symptoms related to hemolysis include back and abdominal pain; headache; smooth muscle dystonias, such as esophageal spasm and erectile dysfunction (due to scavenging of nitric oxide by free plasma hemoglobin); and severe fatigue often out of proportion to the degree of anemia. Exacerbations of hemolysis can occur with infections, surgery, or transfusions and manifest as acute worsening of anemia. If severe, hemolysis can result in acute renal failure because of pigment nephropathy. Icterus often is present intermittently and typically worsens during hemolytic exacerbations.

The most concerning complication of PNH is thrombosis. It is the leading cause of death in the disease. Thrombosis may occur at any site in PNH: venous or arterial. Common sites include intra-abdominal (hepatic, portal, splenic, or mesenteric) and cerebral (cavernous or sagittal sinus) veins, with hepatic vein thrombosis (also known as Budd-Chiari syndrome) being the most common. Deep venous thrombosis, pulmonary emboli, and dermal thrombosis are also prevalent. For unclear reasons, thrombotic complications are less common in PNH patients of Asian descent. The thrombotic propensity is particularly enhanced during pregnancy. Clinically, the complication of

thrombosis is more prevalent in patients as the PNH clone increases in size. Thrombosis may occur in any PNH patient, but those with a large percentage of PNH cells (>50% granulocytes) are at greatest risk. Complement inhibition with eculizumab is the most effective means to stop thrombosis in PNH.

Patients with PNH suffer from anemia but may also have other cytopenias depending on the degree of the associated marrow failure. The marrow failure component of PNH can vary from subclinical disease to SAA and may be categorized as an overlap syndrome of AA/PNH. The disease presentations of PNH and AA do have considerable overlap, as they may represent different spectrums of the same disorder. The PNH clone is often considered a marker of an immune form of marrow failure, as it may predict response to IST in AA; therapies directed at PNH hemolysis will not improve the patient's component of underlying marrow failure.

Treatment

The variability in the clinical manifestations of PNH makes it necessary to individualize the treatment plan. Anemia is often the dominant issue to be addressed. Anemia resulting from hemolysis should be distinguished from BMF-related anemia. Chronic hemolysis should be treated with supportive measures, such as transfusions, supplementation of folate and iron, and, in the context of renal failure, recombinant erythropoietin administration. Table 19-4 outlines standards for clinical care for these patients.

A humanized monoclonal antibody to the C5 terminal complement component, eculizumab, has shown efficacy in decreasing intravascular hemolysis, decreasing the need for transfusions, and improving the quality of life in patients with PNH. Eculizumab effectively stops hemolysis and alleviates the need for transfusions in the majority of patients. It is the only Food and Drug Administration (FDA)-approved therapy for PNH. Treatment with eculizumab is associated with few complications, but because the terminal components of complement are important to protect from *Neisseria meningitidis*, vaccination against this microorganism is important before initiation of eculizumab therapy (at least 2 weeks in advance). The decision about when to start eculizumab needs to take into consideration the degree of chronic hemolysis, frequency of acute hemolytic attacks, severity of constitutional symptoms, thrombotic history, and frequency of transfusions—parameters that should be balanced against the need for chronic lifelong biweekly infusions and the high cost of the drug.

If a diagnosis of a thrombosis is made in a PNH patient, aggressive treatment is warranted. Anticoagula-

Table 19-4 Clinical care of PNH patients

Diagnosis	PNH by FLAER assay LDH Reticulocyte count Complete blood count (CBC)
Therapy	Eculizumab intravenously Loading: 600 mg weekly × 4 weeks Maintenance (followed 1 week later): 900 mg every 2 weeks thereafter Modification to frequency or dose can be considered if ongoing hemolysis Consideration of HSCT in suboptimal responders
Monitoring while on therapy	At least monthly LDH, reticulocyte count, CBC, chemistries At least yearly PNH by FLAER assay If concern for extravascular hemolysis Direct antiglobulin test

tion and eculizumab are indicated for acute thrombotic events; however, primary prophylactic anticoagulation has not been well established to be beneficial in PNH. Anticoagulation after the acute event in a PNH patient well maintained on eculizumab may not be necessarily lifelong.

The majority of classical PNH patients will respond to eculizumab; however, the hemoglobin response is highly variable and may depend on underlying BMF, concurrent inflammatory conditions, genetic factors, and the size of the PNH red cell clone following therapy. Patients do require close monitoring while on eculizumab treatment. Unfortunately, not all patients have their disease-specific needs met by eculizumab. Eculizumab does not improve underlying BMF. There are also reports of patients who have a coexistent autoimmune disease with ongoing activation of complement from their underlying disease, which leads to suboptimal responses from eculizumab. Transient breakthrough intravascular hemolysis can be observed following viral or bacterial infections. Pregnancy can be another limitation on the efficacy of eculizumab. Pregnancy is a hypercoagulable state itself, and there have been concerns both about the potential for increased maternal and fetal morbidity in a pregnant patient as well as the safety of eculizumab therapy in pregnancy. There are multiple case reports and case series reporting successful pregnancies in patients on eculizumab. However, what has been observed is the tendency for breakthrough hemolysis at later stages of pregnancy that requires reduced

dosing interval by the third trimester. Japanese patients can be another group of suboptimal responders to eculizumab. They may carry a single missense C5 heterozygous mutation, c.2654G→A, which prevents binding and blockade by eculizumab while retaining the functional capacity to cause hemolysis. The polymorphism accounts for the poor response to eculizumab in patients carrying the mutation. Lastly, eculizumab only compensates for the CD59 deficiency on PNH erythrocytes, but not the CD55 deficiency. Thus, PNH patients on eculizumab accumulate C3 fragments on their CD55-deficient red cells, leading to extravascular hemolysis through the accumulation of opsonins that are recognized by the reticuloendothelial system (Figure 19-6). Laboratory evidence of extravascular hemolysis in eculizumab-containing patients includes increased reticulocytes, persistent anemia,

and often direct antiglobulin testing that is positive for C3 deposition. These patients may remain asymptomatic, but others have symptomatic anemia and remain dependent on transfusions. Thus, there is a need for complement inhibition that reduces C3 accumulation on PNH erythrocytes to address the shortcomings of eculizumab in PNH (see video on PNH in online edition).

Life-threatening and fatal meningococcal infections have occurred in patients treated with eculizumab due to the complement blockade and inability to fight encapsulated pathogens. Because these infections can be life threatening or fatal, the recommendation is for meningococcal vaccination at least 2 weeks prior to administering the first dose of eculizumab. In patients where the risks of delaying eculizumab therapy outweigh the risk of developing a meningococcal infection, a fluoroquinolone (ciprofloxacin) can be given as a bridge. Furthermore, any infection can increase complement and increase hemolysis, even in patients well managed with stable eculizumab dosing. Attention and increased vigilance at the time of infection are imperative in these patients. Instructions to notify providers for fevers, headaches, or other symptoms should be provided to all patients so that prompt medical attention is available.

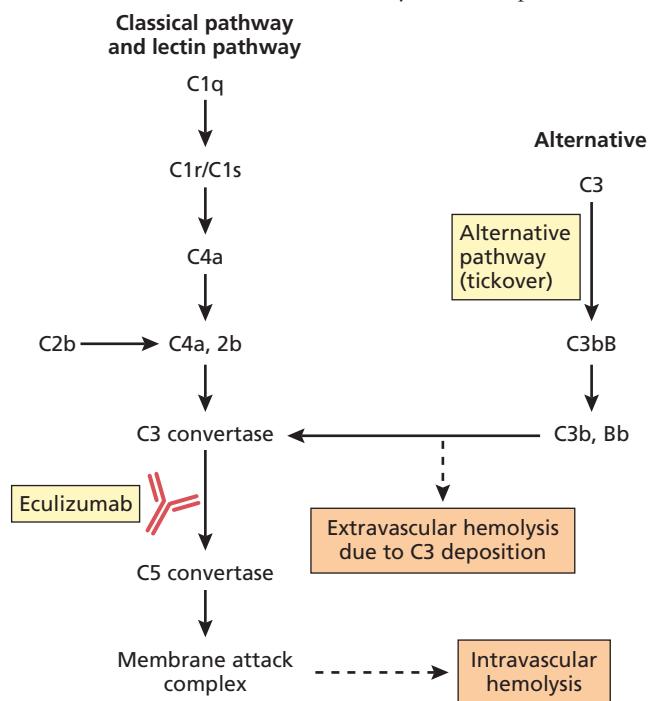
The approach to severe BMF associated with PNH should be similar to that taken for SAA. IST with h-ATG and cyclosporine can be effective in improving blood counts and may allow for better compensation of hemolysis. Immunosuppressive drugs, however, are mostly ineffective in patients with purely hemolytic forms of PNH who have adequate marrow reserve.

HSCT is the only curative therapy for PNH. However, it is not recommended as upfront therapy in the eculizumab era given the risks of transplant-related morbidity and mortality. HSCT is a reasonable therapeutic option in patients who do not respond to therapy with eculizumab or those patients who have severe pancytopenia due to underlying BMF. The transplant paradigm pursued is often with reduced-intensity conditioning regimens, as myeloablation is not required to eradicate the PNH clone. The use of HSCT may be revisited in the future as patients and healthcare providers weigh the cost-benefit ratio of HSCT versus a lifetime of eculizumab therapy.

Prognosis

Thrombotic events, progression of the marrow failure component, and age >55 years at diagnosis have been correlated with a poorer prognosis for PNH patients. The clonal evolution of PNH to MDS or acute leukemia markedly shortens survival. Patients diagnosed with classical PNH without leukopenia, thrombocytopenia, or other

Figure 19-6 The complement cascade, paroxysmal nocturnal hemoglobinuria, and eculizumab. PNH cells have a deficiency in GPI-anchored proteins on their cell surface. Absence of CD55 and CD59 leads to uncontrolled complement activation on the surface of PNH cells. Deficiency of CD59 increases MAC formation and induces intravascular hemolysis, which is central to the pathophysiology of PNH. Deficiency of CD55 leads to increased C3 convertase activity and C3d-associated extravascular hemolysis. Eculizumab therapy for PNH is a humanized monoclonal antibody that targets C5. By preventing C5 activation, eculizumab prevents the formation of the MAC, leading to a significant reduction in intravascular hemolysis of PNH cells. Use of eculizumab can lead to increased extravascular hemolysis in some patients.



complications maintained on therapy can anticipate long-term survival.

KEY POINTS

- Eculizumab, a monoclonal antibody against the C5 terminal complement component, effectively blocks hemolysis in patients with symptomatic PNH and alleviates the need for transfusions in most cases. Eculizumab also appears to reduce thrombotic events. There are limitations to this treatment in some patients, including breakthrough hemolysis and risk of meningococcal infections.
- Prompt evaluation of PNH patients is indicated when symptoms are suggestive of thrombosis because the risk of clotting is high.
- Treatment of bone marrow aplasia with IST will not eliminate the PNH clone and is generally ineffective in primary hemolytic PNH. Immunosuppression, however, may be helpful in patients with AA/PNH syndrome.
- Allogeneic HSCT has curative potential but is indicated only in patients with severe cytopenias and severe thrombotic complications refractory to medical therapy.

Among the potential peripheral blood cytopenias, anemia (often macrocytic) is the most commonly observed cytopenia in MDS, present in >90% of cases at diagnosis. So-called dysplastic cell morphology (discussed in “Diagnostic evaluation” later in this chapter) is diagnostically important, reflects failure of cells to differentiate and mature normally, and often is accompanied by cellular dysfunction that exacerbates the signs or symptoms of cytopenias. For example, hypogranular neutrophils with impaired bactericidal activity compound the infection risk associated with neutropenia, whereas platelets that lack intracellular granules or express abnormally low levels of procoagulant cell surface markers may be ineffective in achieving hemostasis, even when otherwise adequate values of these “dud” cells are present. As a result, the infection and bleeding risks in MDS correlate poorly with the circulating neutrophil and platelet count, and some MDS patients with severe cytopenias are less symptomatic than others with more modest cytopenias. The bone marrow in MDS usually is normocellular or hypercellular for age, but 10% to 20% of cases are accompanied by a hypocellular marrow, and such cases of “hypoplastic MDS” or “hypocellular MDS” may be difficult to distinguish from AA.

Myelodysplastic syndromes

Introduction

MDS include a heterogeneous group of clonal, acquired disorders characterized by ineffective hematopoiesis, resulting in peripheral blood cytopenias. MDS carry a variable risk of progression to acute myeloid leukemia (AML). AML is defined by the World Health Organization (WHO) as >20% blast cells in the marrow or blood, or the presence of certain AML-defining karyotypes such as t(15;17); thus, all patients with MDS have <20% marrow blasts, by definition.

MDS may arise *de novo*—80% to 85% of cases are idiopathic—or may be secondary to a recognized exposure to a DNA-damaging agent. Secondary or therapy-related MDS (t-MDS) can be induced by drugs that alkylate DNA bases (eg, chlorambucil, cyclophosphamide, melphalan), inhibitors of topoisomerase II (eg, topotecan, etoposide, anthracyclines), therapeutic or accidental exposure to ionizing radiation, or environmental or occupational exposure to other DNA toxins, such as hydrocarbons. Proving a causal connection between a suspected exposure and subsequent development of MDS can be challenging, but the presence of a relevant history with a complex karyotype (defined as at least three acquired chromosome abnormalities), abnormalities of chromosomes 5 and 7, or somatic TP53 mutation is suggestive of t-MDS.

Premalignant conditions

Cytopenia is the *sine qua non* for any MDS diagnosis; however, there are individuals with blood cytopenias who do not meet the diagnostic criteria for MDS. Moreover, with the recent advent of inexpensive genomic sequencing technologies, it has also become clear that there are individuals with or without cytopenias who possess somatic clonal mutations known to be associated with MDS such as DNMT3A, TET2, and ASXL1 but do not fully meet WHO criteria for a specific disease entity. Some, but not all, of these individuals will go on to develop MDS or another hematologic neoplasm and will do so at a rate similar to that observed with other premalignant conditions such as monoclonal gammopathy of undetermined significance (a precursor state for plasma cell dyscrasias) and monoclonal B-cell lymphocytosis (a precursor state for B-cell malignancies). The factors that determine progression are not currently well understood, but are thought to involve progressive accumulation of genetic events (Figure 19–7). Due to their different prognoses, it is important to distinguish individuals with these premalignant conditions from those that meet diagnostic criteria for MDS (see video on MDS in online edition). In general, individuals diagnosed with these conditions should be monitored in a proactive fashion for the development of MDS or another hematologic disorder. The following terms have been proposed to describe individuals with cytopenias, clonal

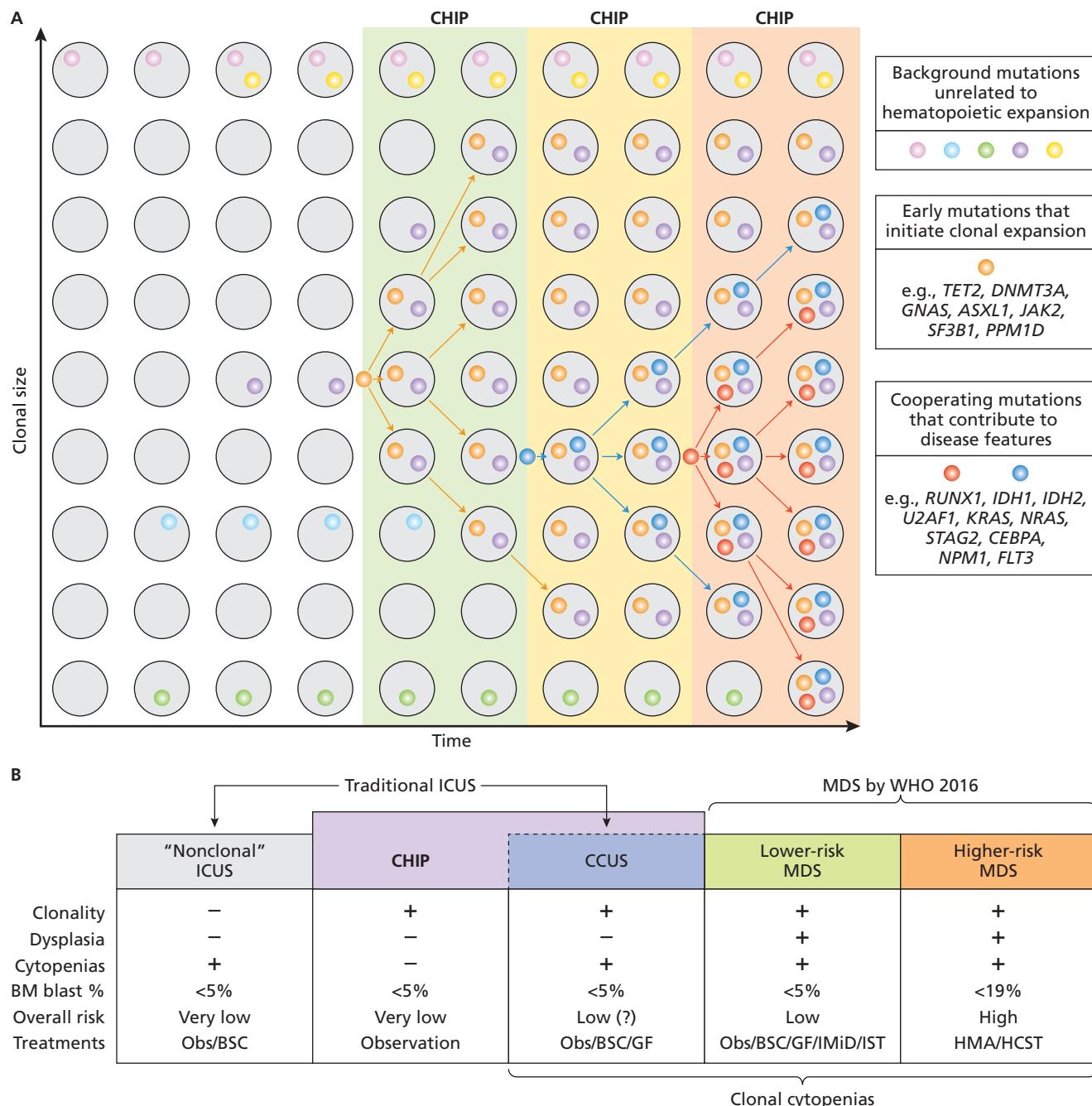


Figure 19-7 Clonal hematopoiesis as a precursor state for hematological neoplasms. (A) A model for evolution from normal hematopoiesis to CHIP and then, in some cases, to MDS or AML. (B) The spectrum of clonal hematopoiesis, ICUS, and MDS. ICUS is a broad category that includes a heterogeneous group of individuals, some of whom have benign (nonclonal) hematopoiesis. Other patients with ICUS may have CHIP, differing only from lower-risk MDS by their lack of dysplasia and, currently, an undetermined disease risk. CHIP can also include patients with clonal hematopoiesis and nonmalignant causes of cytopenias (eg., immune cytopenias, liver disease, or nutritional deficiencies) that would not be considered to have ICUS because of the presence of a clone, but may have a distinct natural history. BM, bone marrow; BST, best supportive care; GF, hematopoietic growth factor (eg., epoetin); HMA, hypomethylating agent (eg., azacitidine); IMiD, immunomodulatory drug (eg., lenalidomide); Obs, observation. Adapted from Steensma DP et al, *Blood*. 2015;126:9–16.

mutations seen in myeloid neoplasms, or both who do not meet formal WHO criteria for MDS:

ICUS: Individuals with single or multiple blood cytopenias that remain unexplained despite an appropriate evaluation (including bone marrow examination) and do not have a known associated clonal genetic alteration. Individuals with ICUS may have cytopenias due to undiagnosed reactive condition, other nonneoplastic conditions or a nonmyeloid neoplasm.

CHIP: Individuals known to have a clonal mutation associated with hematologic neoplasia but do not yet meet diagnostic criteria for diagnosis of any hematologic neoplasm and do not have a clinically significant cytopenia. The risk of CHIP increases with age, occurring in >10% of individuals over age 70 years with normal blood counts, and patients with prior exposure to chemotherapy or radiation appear to have higher rates of CHIP compared to a noncancer population. Individuals with CHIP have an increased risk of progression to a hematologic malignancy that is estimated at 0.5% to 1% per year. CHIP is also associated with an increase in all-cause mortality and an increased risk of cardiovascular events. This is currently attributed to the concept that the clonally derived cells further promote inflammation in atherosclerotic plaques.

CCUS: Individuals with a clonal mutation and one or more clinically meaningful unexplained cytopenias who do not meet WHO-defined criteria for a hematologic neoplasm. The progression risk for CCUS to overt MDS is higher than for ICUS or CHIP.

Classification

The WHO classification of MDS was revised in 2016 (Table 19-5) as part of an overall revision to the WHO classification of myeloid neoplasms and acute leukemia. The 2016 WHO MDS classification was a minor revision of the classification from the fourth edition of the WHO *Classification of Tumors of Hematopoietic and Lymphoid Tissues*, published in 2008. This update was intended to incorporate discovery of newly identified molecular features that have provided diagnostic and prognostic information as well as pathological insights into MDS disease biology.

Important classification factors in the current WHO MDS schema include the number of lineages with dysplasia in >10% of cells, the marrow and peripheral blood blast proportion (determined as a percentage of all nucleated bone marrow cells), whether or not <15% of erythroid precursor cells in the marrow are ring sideroblasts (or <5% if *SF3B1* mutation is present); whether or not Auer rods are present, and the presence of disease-defining cytogenetic abnormalities. Despite the discovery of recurrent mutations that can be identified in 80% to 90% of MDS

patients, the WHO has incorporated only recurrent mutations in the spliceosome gene *SF3B1* into the diagnostic scheme of MDS with ring sideroblasts (MDS-RS). This is based on the clear link between ring sideroblasts and *SF3B1* mutation and that these cases are associated with a distinct gene expression profile and favorable prognosis. It is important to reiterate that the presence of MDS-related mutations alone (with absence of morphologic dysplasia), even in the presence of clinically significant cytopenias, is not diagnostic of MDS. Such individuals may still have an unrelated reactive cause of cytopenia and are best monitored as CHIP or CCUS. The WHO has grouped t-MDS with therapy-related AML because the outcome in such patients is poor, regardless of the blast count. Cases with both MDS and myeloproliferative features, such as leukocytosis or thrombocytosis, are classified in a separate “overlap” category of MDS/myeloproliferative neoplasms (MPNs), which includes chronic myelomonocytic leukemia (defined by $\geq 1 \times 10^9/L$ blood monocytes) and MDS/MPN with ring sideroblasts and thrombocytosis (which requires a platelet count $\geq 450 \times 10^9/L$). Although the WHO classification is useful diagnostically, it has only limited prognostic value, and other tools (described below) are more useful for risk stratification.

The observation that alkylating agents, topoisomerase inhibitors, and ionizing radiation predispose patients to both MDS and AML; evolution of MDS to AML in some patients over time; the existence of shared cytogenetic abnormalities, such as deletions or gains in all or parts of chromosomes 5, 7, 8, or 20; and shared common somatic mutations, such as *TET2* and *ASXL1*, imply a biologic continuum between MDS and AML. Whereas loss or gain of chromosomal material is common in MDS, chromosomal translocations are less common in MDS than in AML, and certain point mutations (eg., *FLT3*) common in AML are rarely seen in MDS. The so-called “good-risk” recurrent AML-associated translocations, t(8;21), t(15;17), and inv(16), are rare in patients with dysplasia, and the WHO classifies patients with these abnormalities as having AML regardless of the blast count or marrow dysplasia.

The natural history of MDS includes a risk of progression to treatment-refractory AML (~25% to 30% likelihood overall, with some subtypes of MDS such as MDS with excess blasts [MDS-EB-2] at much greater risk), but most patients with MDS do not develop AML. Instead, the majority of patients who are diagnosed with MDS will die from complications of cytopenias, most commonly infections resulting from absolute neutropenia and neutrophil dysfunction, and less frequently thrombocytopenia-associated bleeding or anemia-exacerbated cardiovascular events. Because MDS are primarily diseases of older persons,

Table 19-5 2016 WHO classification of myelodysplastic syndromes and neoplasms

Name	Dysplastic lineages	Cytopenias*	Bone marrow (BM)/peripheral blood (PB) features	Cytogenetics by conventional karyotype analysis
MDS with single lineage dysplasia	1	1 to 2	Blasts: BM < 5%, PB < 1%, no Auer rods; $<15\%/\text{PB } < 5\%$ † ring sideroblasts	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia	2 or 3	1 to 3	BM < 5%, PB < 1%, no Auer rods; $<15\%/\text{PB } < 5\%$ † ring sideroblasts	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS				
MDS-RS with single lineage dysplasia	1	1 to 2	BM < 5%, PB < 1%, no Auer rods; $<15\%/\text{PB } < 5\%$ † ring sideroblasts	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia	2 or 3	1 to 3	BM < 5%, PB < 1%, no Auer rods; $<15\%/\text{PB } < 5\%$ † ring sideroblasts	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1 to 3	1 to 2	BM < 5%, PB < 1%, no Auer rods; del(5q) alone or with one additional abnormality except -7 or del(7q); no ring sideroblasts	del(5q) alone or with one additional abnormality except -7 or del(7q)
MDS-EB				
MDS-EB-1	0 to 3	1 to 3	BM 5% to 9% or PB 2% to 4%, no Auer rods; no ring sideroblasts	Any
MDS-EB-2	0 to 3	1 to 3	BM 10% to 19% or PB 5% to 19% or Auer rods; no ring sideroblasts	Any
MDS, unclassifiable				
With 1% blood blasts	1 to 3	1 to 3	BM < 5%, PB = 1%‡ no Auer rods; no ring sideroblasts	Any
With single lineage dysplasia and pancytopenia	1	3	BM < 5%, PB < 1%, no Auer rods; no ring sideroblasts	Any
Based on defining cytogenetic abnormality	0	1 to 3	BM < 5%, PB < 1%, no Auer rods; $<15\%§$	MDS-defining abnormality
Refractory cytopenia of childhood	1 to 3	1 to 3	BM < 5%, PB < 2%, no ring sideroblasts	Any

Adapted from Arber DA et al. *Blood*. 2016;127(20):2391–2405.

*Cytopenias defined as: hemoglobin, <10 g/dL; platelet count, <100 × 10⁹/L; and absolute neutrophil count, <1.8 × 10⁹/L. PB monocytes must be <1 × 10⁹/L.

†If *SF3B1* mutation is present.

‡One percent PB blasts must be recorded on at least two separate occasions.

§Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia and are classified as MDS-RS with single lineage dysplasia.

—MDS-defining abnormalities (by conventional cytogenetics): -7 or del(7q), t(11;16)(q23;p13.3), -5 or del(5q), t(3;21)(q26.2;q22.1), i(17q) or t(17p), t(1;3)(p36.3;q21.1), -13 or del(13q), t(2;11)(p21;q23), del(11q), inv(3)(q21q26.2), del(12p) or t(12p), t(6;9)(p23;q34), del(9q), idic(X)(q13), or complex karyotype (three or more chromosomal abnormalities involving one or more of the above).

some patients succumb to unrelated conditions that are common in the elderly; they die with MDS, rather than from MDS.

Epidemiology

Aging is the most important risk factor for development of MDS, in part because of the progressive accumulation of somatic mutations in HSCs across the human life span. Eventually, a mutation or combination of mutations can occur in such a way in a hematopoietic cell that its progeny acquires a growth and survival advantage and clonal hematopoiesis emerges. The expanded clone of cells is then at

risk for acquiring additional mutations that increase its malignant potential.

The median age at diagnosis of MDS in the United States and Europe is ~70 years. In China and Eastern Europe, the median age at diagnosis is more than a decade younger than in the West, possibly due to environmental factors. Overall, there is a slight male predominance in MDS that may be related in part to occupational exposures, but this imbalance may also have a biological basis related to a protective effect of having two X chromosomes. However, one specific MDS subtype, MDS associated with isolated deletion of the long arm of chromosome 5 and a marrow

morphology that includes hypolobated megakaryocytes and erythroid hypoplasia (ie., 5q⁻ syndrome), is more common in women than in men.

Accurate estimates of the incidence of MDS have been difficult to obtain because MDS cases have not historically been captured by cancer registries and many elderly patients with mild cytopenias are incompletely evaluated. However, current registry and claims-based algorithms suggest there are 30,000 to 40,000 new cases of MDS diagnosed per year in the United States. Most patients have lower-risk disease at the time of initial diagnosis.

MDS diagnoses are rare in the pediatric age group and represent ~5% of hematologic malignancies in patients <18 years of age. When MDS does arise in children, the diagnosis is frequently associated with Down syndrome, congenital marrow failure syndromes, or germ-line defects of DNA repair, such as LiFraumeni syndrome or Bloom syndrome. Children with Shwachman-Diamond syndrome, congenital neutropenia, or Fanconi anemia (FA) are at markedly increased risk of developing MDS (see Chapter 16). In all of these inherited conditions, MDS arises in the context of hematopoietic deficits and typically presents in late childhood or in adolescence. Children who develop MDS without excess blasts but who appear to lack a predisposing congenital syndrome are provisionally classified by the WHO as having refractory cytopenia of childhood.

MDS with excess blasts is also relatively uncommon in children, and the bone marrow is often hypocellular rather than the hypercellular marrow characteristic of adults; there is also a high incidence of unfavorable biologic features, such as monosomy 7. MDS-RS and 5q⁻ syndrome are rare in children, although a number of forms of congenital sideroblastic anemia can be confused with MDS, such as sideroblastic anemia due to germ-line mutations of *ALAS2*, which does not carry a risk of progression to AML.

In the majority of adult patients with MDS, the etiology is unknown, and there is no specific predisposing factor identifiable other than advanced age. However, a subset of patients with MDS, AML, or MPN, particularly those with a family history of related disorders/cytopenias or with t-MDS, may have a familial syndrome with inherited germ-line predisposition. The 2016 revision of the WHO classification now identifies these cases as myeloid neoplasms with germline predisposition. Examples of these include myeloid neoplasms associated with germline mutations in *RUNX1* and *GATA2* transcription factors or mutations in genes with less understood function such as *ANKRD26* and *DDX41*. Germline *RUNX1* and *ANKRD26* mutations are associated with a prodrome of thrombocytopenia, whereas *DDX41* mutations have no associated prodrome.

GATA2 mutations are sometimes nonsyndromic but can be associated with mycobacterial infections, lymphedema, and monocytopenia (MonoMAC syndrome).

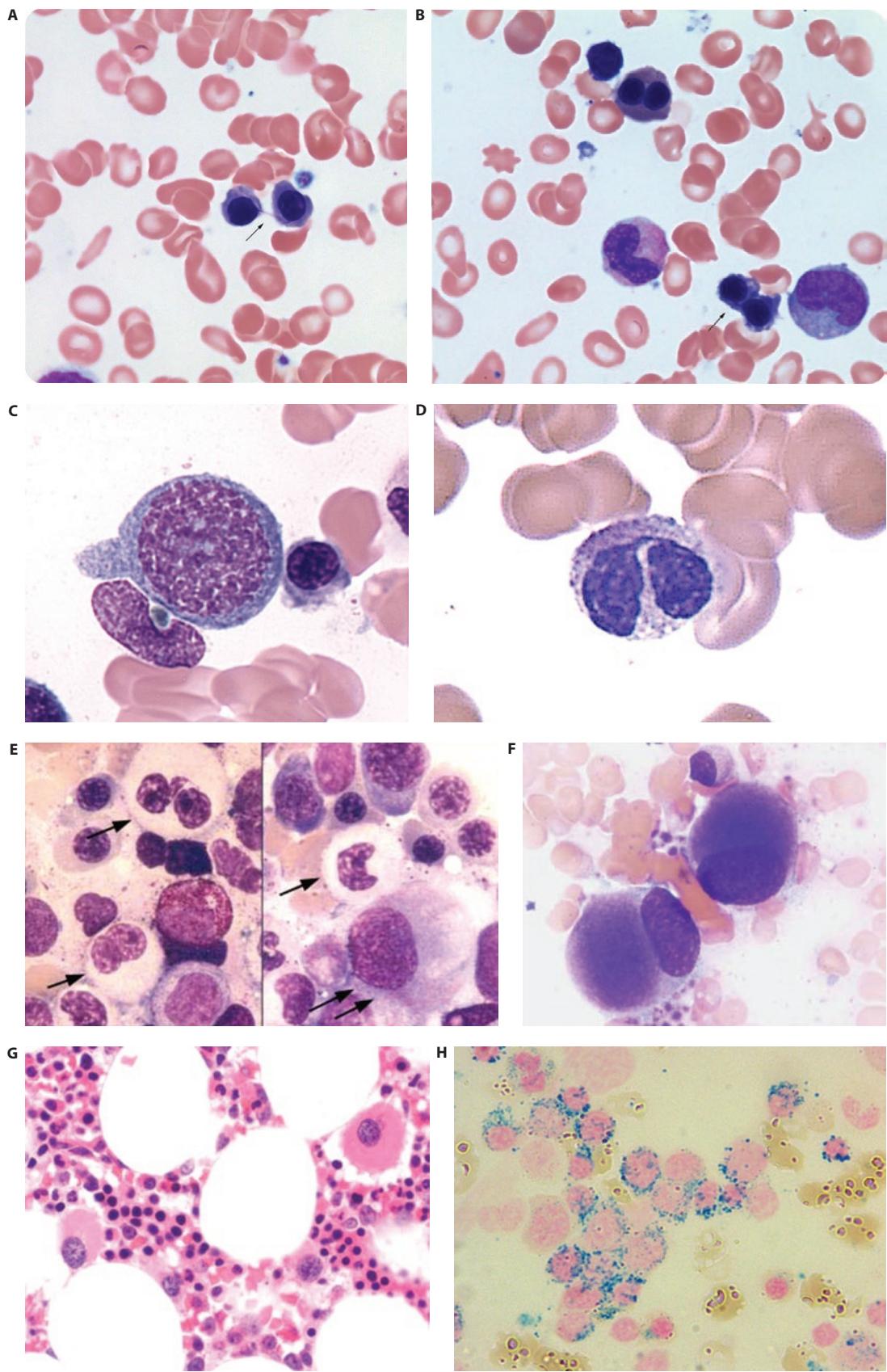
KEY POINTS

- MDS is characterized by ineffective hematopoiesis, leading to peripheral blood cytopenias. The marrow is often hypercellular for age.
- Anemia (usually macrocytic) is the most common cytopenia associated with MDS. Functional defects in neutrophils and platelets can exacerbate the risk of infection from neutropenia or bleeding from thrombocytopenia.
- Aging and exposure to alkylating agents, topoisomerase II inhibitors, or ionizing radiation are all risk factors for developing MDS.
- MDS is rare in children, and when they occur are often associated with congenital marrow failure syndromes.
- An increasing number of germ-line mutations such as those in *RUNX1* and *GATA2* are associated with a subsequent risk for MDS development. *RUNX1* mutations are also associated with thrombocytopenia and are often mistaken for immune thrombocytopenic purpura (ITP) until MDS develops or additional family members are diagnosed.
- The 2016 WHO classification of MDS is the current standard, but it should be used in conjunction with risk stratification tools to assess prognosis.

Diagnostic evaluation

After a medical history and physical examination, the diagnosis of MDS is readily established in most patients by a complete blood count, careful review of the blood smear, bone marrow examination, and basic laboratory tests to rule out other disorders that mimic MDS. Vitamin B₁₂ and folate deficiency, HIV infection, copper deficiency, alcohol abuse, and adverse effects of medication (eg., antimetabolites such as methotrexate or azathioprine) need to be excluded as should other causes of anemia such as iron deficiency and thyroid disorders. Cytopenias should be persistent (at least 4 to 6 months in duration) and cannot be attributable to other underlying conditions. The pathologic diagnosis of MDS currently emphasizes morphologic criteria demonstrating dysplastic features in the peripheral blood and >10% of bone marrow precursor cells in one or more lineages—erythroid, myeloid, megakaryocytic (Figure 19-8). Additionally, an increased blast count (5% to 19%) or presence of an MDS-associated karyotype is also diagnostic.

In one large study, the median hemoglobin of patients diagnosed with MDS was 9.5 g/dL, and 75% of



patients had a level of <11 g/dL. Only 20% of patients had both a platelet count $>100 \times 10^9/\text{L}$ and an absolute neutrophil count $>1.0 \times 10^9/\text{L}$ at diagnosis, indicating that a presentation with anemia alone in MDS is relatively uncommon. Although patients with MDS often seek medical attention because of symptoms related to cytopenias, especially fatigue or poor exercise tolerance, many patients are asymptomatic at diagnosis and are discovered to have MDS only when a complete blood count is performed as a screening test or to evaluate another condition.

Oval macrocytic red blood cells, hypogranular and hypolobulated granulocytes, and giant or hypogranular platelets can be identified in the peripheral blood of many patients with MDS. Bilobated hyposegmented neutrophils in MDS resemble those seen in the clinically inconsequential congenital Pelger-Huët anomaly and are referred to as Pelgeroid or pseudo-Pelger-Huët cells. Peripheral blood smears may be highly suggestive of the diagnosis, but are never conclusive by themselves. A marrow aspirate is essential to establish definitively a diagnosis of MDS, and the bone marrow core biopsy provides complementary information on cellularity and architecture, megakaryocyte morphology, and the presence of fibrosis—useful information that may inform therapeutic decisions.

The bone marrow biopsy in MDS usually demonstrates hypercellularity, which, in the setting of cytopenias in the peripheral blood, indicates ineffective hematopoiesis. On the marrow aspirate, megaloblastoid red blood cell precursors with asynchronous maturation of the nucleus and the cytoplasm are usually evident, and multinucleated erythroid precursors are common (Figure 19-8). Ring sideroblasts, which are erythroid precursors with iron-stuffed mitochondria (stored as mitochondrial ferritin, a unique type of ferritin) surrounding at least one-third of the nucleus, may be identified via the Prussian blue reaction, and

often there is predominance of immature myeloid cells and dysplastic granulocytic precursors. Dysplastic megakaryocytes may be smaller or larger than normal and may be hypolobated or hyperlobated. Dysplastic features in all lineages can include nuclear and cytoplasmic blebs and misshapen nuclei.

Cytogenetic studies can further support a diagnosis of MDS, and are important for prognosis and treatment decisions (Tables 19-6, 19-7, and 19-8). Standard cytogenetic assessment is preferred, but in a small percentage of cases, fluorescence *in situ* hybridization (FISH) analysis with probes directed towards chromosomes frequently rearranged in MDS (eg, 5, 7, 8, 20) reveals specific chromosomal translocations and losses or gains of DNA segments that were not detected with standard cytogenetic methods. FISH is helpful in cases in which 20 or more metaphases cannot be obtained, but the yield of FISH is low if karyotyping is successful. The clinical relevance of small clones detectable only by FISH is uncertain.

Flow cytometric analysis of the bone marrow, which is now a standard procedure for diagnosing and subclassifying patients with acute leukemia, is being used increasingly to evaluate patients suspected of having MDS. It is still considered nonessential, but a number of investigative groups have described abnormal cell populations and inappropriate antigen expression detected by flow cytometry, and these investigators continue to study the diagnostic specificity and prognostic importance of specific flow findings on a research basis. Flow cytometry can also be helpful to detect clonal expansion of large granular lymphocytes, which may predict response to IST. Because accurate classification according to WHO criteria is based, at least in part, on bone marrow morphology, flow cytometry should be viewed as a complementary test that is best interpreted in the context of the appearance of the marrow morphology. Specifically, flow cytometric enumeration of marrow blasts should not replace a manual

Figure 19-8 Typical blood and marrow cell morphology in patients with MDS. (A and B) Multinucleated erythroid precursors (arrows); the cells in panel A have a visible cytoplasmic bridge, which is uncommonly observed. Wright-Giemsa-stained marrow aspirate. Source: ASH Image Bank (imagebank.hematology.org), #00030315. (C) Megaloblastoid erythroid cell maturation (nuclear-cytoplasmic dys-synchrony). The chromatin pattern of these cells is fine, suggesting relative immaturity, whereas the lightening of the cytoplasm indicative of early hemoglobinization is an event typically associated with later stages of maturation. Source: ASH Image Bank #00002571. (D) Hypolobated neutrophil (pseudo-Pelger-Huët cell) found in the peripheral blood of a patient with refractory cytopenias with multilineage dysplasia. The cell vaguely resembles a pince-nez, a style of eyeglasses popular in the 19th century supported without earpieces. Source: ASH Image Bank #00002117. (E and F) Hypogranular neutrophils (arrows). These would be expected to have poor bactericidal activity. The double arrow in panel F indicates a small, dysplastic megakaryocyte. Source: ASH Image Bank #00001435. (G and H) Micromegakaryocytes in a Wright-Giemsa-stained aspirate (G) and hematoxylin-eosin-stained core trephine biopsy specimen (H). These may have an eccentric, hypolobulated, or round nucleus. These images are from a patient with 5q- syndrome. Source: ASH Image Bank #00001446 (H) and #00001448 (G). (I) Ring sideroblasts (a Prussian blue reaction on a marrow aspirate, seen at low power magnification and counterstained with neutral red). Source: ASH Image Bank #00001157.

Table 19-6 The 1997 IPSS for myelodysplastic syndromes

Prognostic factor	Category score (sum all three subscores for overall IPSS score)				
	0 (best)	0.5	1	1.5	2.0 (worst)
Marrow blasts (%)	<5	5 to 10	—	11 to 20	21 to 30*
Karyotype	Good: normal, isolated -Y, isolated del(5q), or isolated del(20q)	Intermediate: all karyotypes not defined as good or poor	Poor: abnormal chromosome 7 or a complex karyotype (≥ 3 anomalies)	—	—
Peripheral blood cytopenias†	0 or 1	2 or 3	—	—	—

Scoring system: A point value from 0 to 2.0 is determined for each of the three prognostic factors in Table 19-6, and the three values are summed to obtain the total IPSS score (see Table 19-7).

While replaced by the IPSS-R in 2012 (see below), numerous clinical trial protocols still use the original IPSS for determination of eligibility. From Greenberg P et al, *Blood*. 1997;89:2079–2088.

*No longer considered myelodysplastic syndrome (redefined as acute myeloid leukemia by WHO in 2001).

†IPSS definition of peripheral blood cytopenias: hemoglobin, <10 g/dL; absolute neutrophil count, $<1.8 \times 10^9/L$; and platelet count, $<100 \times 10^9/L$.

Table 19-7 Risk stratification of IPSS

Risk category	Total score	Median survival (years)	Median survival (years) for patients <60 years old (n = 205)	Median survival (years) for patients ≥60 years old (n = 611)	Time until 25% of surviving patients in category developed leukemia (years)
Low risk	0	5.7	11.8	4.8	9.4
Intermediate-1 (INT-1)	0.5 or 1.0	3.5	5.2	2.7	3.3
Intermediate-2 (INT-2)	1.5 or 2.0	1.2	1.8	1.1	1.1
High	2.5	0.4	0.3	0.5	0.2

From Greenberg P et al, *Blood*. 1997;89:2079–2088.

differential from the marrow aspirate, because it is subject to technical artifacts.

Increasingly, molecular profiling is playing an important role in evaluation of patients suspected of having MDS, especially in ambiguous cases with bland morphology but no other explanation for cytopenias. Almost all patients with MDS have a somatic mutation detectable in one of the 25 to 40 most commonly mutated MDS-associated genes, so the negative predictive value of a normal result on an MDS mutation panel is high, and another cause for cytopenias should be carefully sought in such cases. However, as mentioned earlier, because clonal hematopoiesis is common in healthy older people, detection of a mutation in patients with a normal karyotype and without morphological changes of dysplasia should be interpreted with caution and is not diagnostic of MDS. Molecular profiling can also aid in prognostic assessment and decisions about stem cell transplant. Detection of an *SF3B1* mutation, for instance, would support a diagnosis of MDS-

RS rather than a congenital sideroblastic anemia or reactive cause of sideroblastic anemia, while the finding *TP53* mutation makes stem cell transplant less likely to be successful. Finally, additional genetic screening for mutations associated with inherited predisposition syndromes should be strongly considered in patients with a family history of hematologic malignancies and familial cytopenias, in patients with t-MDS, or in younger patients with MDS. Such testing should ideally be done on constitutional tissue such as skin fibroblasts in order to confirm the germline nature of such alterations and avoid false negatives associated with peripheral blood somatic mosaicism.

Overall, the diagnosis of MDS is evolving toward the approach used in AML, in which morphologic, cytogenetic, and flow cytometric data are assessed together to make an accurate diagnosis and determine the optimal treatment. This strategy will become increasingly important as biologically distinct subsets of MDS patients who respond to specific therapies are defined.

Table 19-8 IPSS-R for MDS (2012 version)

Parameter	IPSS-R categories and associated scores				
	Very good	Good	Intermediate	Poor	Very poor
Cytogenetic risk group	0	1	2	3	4
Marrow blast proportion	<2%	2% to <5%	5% to 10%	>10%	
	0	1	2	3	
Hemoglobin	≥10 g/dL	8 to <10 g/dL	<8 g/dL		
	0	1	1.5		
Absolute neutrophil Count	≥0.8 × 10 ⁹ /L	<0.8 × 10 ⁹ /L			
Platelet count	≥100 × 10 ⁹ /L	50 × 10 ⁹ to 100 × 10 ⁹ /L	<50 × 10 ⁹ /L		
	0	0.5	1		

Possible range of summed scores: 0 to 10.

Adapted from Greenberg PL et al, *Blood*. 2012;120:2454–2465.

KEY POINTS

- Complete blood counts, marrow aspirate and core biopsy, blood and marrow morphology, and cytogenetic testing are key. Next-generation sequencing is increasingly used as well to establish a diagnosis of MDS.
- Flow cytometry may provide complementary information but cannot be used to establish a diagnosis of MDS in the absence of marrow morphology. Blast counts should be based primarily on a manual assessment of the marrow aspirate by an experienced morphologist.
- Vitamin B₁₂ and folate deficiency, HIV infection, copper deficiency, alcohol abuse, and medication effects (eg, antimetabolites such as methotrexate) can cause cytopenias and dysplastic changes in blood cells and need to be excluded.
- Molecular abnormalities are present in most cases of MDS, and molecular testing can be used as a supplemental diagnostic tool and as an aid in prognostic assessment.

A major limitation of the 1997 IPSS is that it does not distinguish between patients with severe and modest degrees of cytopenias, which may influence outcome. For example, a platelet count of 9 × 10⁹/L is not weighted any differently by the IPSS than a count of 90 × 10⁹/L, although several studies have shown that severe thrombocytopenia is an important risk factor for disease progression and death. The IPSS is valid only for patients with *de novo* disease treated with supportive care and is not useful during the course of the disease or in previously treated patients; within each IPSS risk group, there are wide variations in patient outcomes. Despite these shortcomings, the IPSS has greater prognostic value than the WHO classification system for individual patients.

Several newer MDS prognostic systems have been introduced since 2007 to try to overcome limitations of the IPSS and are becoming more widely incorporated. These newer risk stratification models include the WHO-based Prognostic Scoring System, which integrates the WHO classification with karyotyping data and the degree of anemia; a modified form of the WHO-based Prognostic Scoring System includes the presence or absence of marrow fibrosis. A general risk model proposed by investigators at the M.D. Anderson Cancer Center in Houston, Texas, is valid across a broad spectrum of MDS patients, including those with exposure-related MDS and those who previously have been treated (eg, with a hypomethylating agent). A risk model specific to lower-risk MDS also was developed at the M.D. Anderson Cancer Center and has been independently validated by other groups.

In 2012, a revised version of the IPSS (IPSS-R) was published, based on analysis of >7,000 patients from more than 10 countries (Tables 19-8, 19-9, and 19-10). The primary

Prognosis

In 1997, the International Prognostic Scoring System (IPSS) (Tables 19-6 and 19-7) was developed to help stratify patients with MDS by their risk of disease progression to acute leukemia and death. The overall IPSS score is based on the sum of three subscores—scores for the karyotype, percentage of bone marrow blasts, and number of qualifying cytopenias. Patients >60 years of age with a low IPSS score have a median survival of 4.8 years, whereas patients in this age group with a high IPSS score have a median survival of only <6 months, if treated with supportive care alone. For each IPSS risk group, outcomes tend to be better for younger patients than for older patients.

Table 19-9 MDS cytogenetic risk stratification system used in the IPSS-R

Risk group	Updated cytogenetic classification for use in IPSS-R (n = 7012)			
	Included karyotypes	Median survival, years	25% of patients to AML, years	Proportion of patients in this group
Very good	del(11q), -Y	5.4	N/R	4%
Good	Normal, del(20q), del(5q) alone or with 1 other anomaly, del(12p)	4.8	9.4	72%
Intermediate	+8, del(7q), i17q, +19, any other single or double abnormality not listed, two or more independent clones	2.7	2.5	13%
Poor	Abnormal 3q, -7, double abnormality include -7/del(7q), complex with three abnormalities	1.5	1.7	4%
Very poor	Complex with more than three abnormalities	0.7	0.7	7%

N/R, not reached.

Adapted from Greenberg PL et al, *Blood*. 2012;120:2454–2465.**Table 19-10** Survival and AML progression risk with the 2012 IPSS-R for MDS

Risk group	Points	% patients (n = 7,012; AML data on 6,485)	Median survival, years	Median survival for patients under 60 years	Time until 25% of patients develop AML, years
Low	2.0 to 3.0	38%	5.3	8.8	10.8
Very low	0 to 1.5	19%	8.8	Not reached	Not reached
Intermediate	3.5 to 4.5	20%	3.0	5.2	3.2
High	5.0 to 6.0	13%	1.5	2.1	1.4
Very high	>6.0	10%	0.8	0.9	0.7

Adapted from Greenberg PL et al, *Blood*. 2012;120:2454–2465.IPSS-R (see: <http://www.mds-foundation.org/ipss-r-calculator/>).

changes in the IPSS-R are that it includes a broader range of cytogenetic abnormalities than the small list of MDS-associated karyotypes that were included in the 1997 IPSS version, and the IPSS-R also weighs cytogenetic findings more heavily than other variables. In addition, degree of cytopenias is given more weight in the IPSS-R than in IPSS, and blast cutoffs are different. Like the original IPSS, however, the IPSS-R is most valid in patients with *de novo* MDS and only at the time of diagnosis. In addition, other prognostically important variables, such as the presence of comorbid conditions and the patient's performance score, molecular genetic findings, and the kinetics of clonal evolution and disease progression, are not accounted for by the IPSS-R or any of the other major prognostic tools. Table 19-10 shows survival estimates.

More than 80% of patients with MDS have at least one somatic mutation detectable in hematopoietic cells (see “Biology” below). Several of these mutations have IPSS-independent prognostic significance. For instance, patients with mutations in *TP53*, *ETV6*, *RUNX1*, *ASXL1*, or *EZH2* have a greater risk of leukemia progression or

death than would be predicted by the IPSS, and patients with IPSS low-risk disease who harbor one of these mutations have an outcome more similar to IPSS intermediate-1-risk disease. New prognostic systems are incorporating molecular abnormalities into the IPSS-R.

KEY POINTS

- The IPSS was previously the most widely used risk stratification system in MDS, but was revised in 2012 (IPSS-R) to include a broader range of karyotypes and other modifications. The IPSS_R is now broadly used to prognosticate for MDS patients.
- Factors associated with poorer outcomes in MDS include advanced age, comorbid conditions and poor performance score, increased marrow and blood blasts, more severe cytopenias and transfusion dependence, higher-risk karyotypes (eg, a complex karyotype or monosomy 7), and the presence of certain mutations (eg, *TP53* or *RUNX1*).

Biology

Chromosome and molecular biology

Approximately one-half of patients with *de novo* MDS and most patients with t-MDS have cytogenetic abnormalities detectable on routine G-banded metaphase karyotyping. Cytogenetic results have independent prognostic significance (Table 19–9). New clonal cytogenetic aberrations emerge in >25% of patients with MDS during the course of their disease, which suggests genomic instability of some form, although microsatellite instability is not common. In patients with MDS who have a normal karyotype, more sensitive analytical techniques, such as single-nucleotide polymorphism arrays and array-based comparative genomic hybridization, frequently detect areas of loss of heterozygosity and uniparental disomy, which often are clonally restricted (ie., not present in germline tissue).

One particular clonal abnormality involving interstitial or terminal deletion of part of the long arm of chromosome 5 (5q-) has received a great deal of attention because patients with deletions of chromosome 5q preferentially respond to lenalidomide therapy (see section “Treatment of MDS” later in this chapter). Haploinsufficiency of a 5q-encoded ribosomal protein, RPS14, contributes to defective erythropoiesis, just as germline mutations of ribosomal components contribute to DBA (see section on DBA in Chapter 16). As originally described, the 5q- syndrome is associated with erythropoietin-refractory macrocytic anemia, dyserythropoiesis, normal or increased platelet count, giant platelets, hypolobated megakaryocytes, variable neutropenia, female predominance, prolonged survival, and a low rate of leukemic transformation. It is important to differentiate the 5q- syndrome from other myeloid disorders in which chromosome 5q deletions are found as they are not biologically the same. Patients with the del(5q) without the characteristic clinical and morphologic features of 5q- syndrome may have a more aggressive clinical course and shorter survival than those with the classic syndrome, although they still may respond to lenalidomide treatment. The extent of the chromosome 5q deletion in MDS also has prognostic value, with small interstitial deletions associated with better outcomes than larger deletions.

The clinical and genetic heterogeneity found in MDS and the typical advanced age at disease onset support the idea that multiple cooperating genetic lesions contribute to leukemogenesis. Unlike AML and MPNs, which frequently demonstrate chromosomal translocations, gains and losses of entire chromosomes (eg, monosomy 5 and 7 or trisomy 8) or of large DNA segments (eg, many megabase pairs of chromosomes 5q, 7q, or 20q) are more

common in MDS, which has made pinpointing individual genes that contribute to the development or progression of MDS via a candidate-gene approach a formidable challenge. In recent years, high-throughput resequencing techniques revealed recurrent point mutations in more than 40 different genes, some of which are shared with AML and other neoplasms (Figure 19–9). These techniques also demonstrate that the majority of cells in the marrow are clonal, even in lower-risk MDS with <5% blasts.

Activating mutations in proto-oncogenes such as *NRAS*, *FLT3*, and *JAK2* are detected in many cases of AML or MPN but are uncommon in MDS. Although RAS/RAF pathway mutations are common in the MDS-MPN overlap syndromes of chronic myelomonocytic leukemia and juvenile myelomonocytic leukemia, these mutations are rare in MDS without MPN features and usually are found only after progression to acute leukemia. These data suggest that aberrant activation of signal transduction pathways may not be a major mechanism of aberrant cell growth and clonal dominance in early MDS, which distinguishes these diseases from other myeloid malignancies.

The *TP53* tumor suppressor gene, which regulates cell cycle progression, DNA repair, and apoptosis, is mutated in 5% to 10% of MDS cases overall and in a higher proportion of t-MDS. *TP53* mutation is often associated with a complex karyotype and has a strong negative prognostic significance. *RUNX1* point mutations also are relatively common in patients with t-MDS.

Mutations in genes altering DNA methylation and chromatin remodeling are common in MDS. *TET2* mutations, for example, are present in 20% to 30% of patients, and recurrent mutations are also found in *EZH2*, *IDH1* and *IDH2*, and *ASXL1*. Another class of recurrent mutations in MDS are those in genes that encode components of the spliceosome and alter RNA splicing, especially *SF3B1*, which is present in the majority of patients with MDS-RS. Other common mutations in spliceosome components include *SRSF2* and *U2AF1*. Mutations in genes such as *STAG2* or *RAD21* that encode components of the cohesin protein complex, which regulates the separation of sister chromatids during cell division, are found in up to 20% of MDS.

Patients who develop t-MDS secondary to exposure to mutagenic or carcinogenic agents almost always have chromosomal abnormalities. t-MDS is most commonly associated with previous treatment with alkylating agents or exposure to ionizing radiation, and these cases frequently demonstrate losses involving chromosomes 5 or 7. The latency period for t-MDS arising after alkylating agent therapy is typically 3 to 7 years. Patients treated with epipodophyllotoxins (eg, etoposide) can develop specific

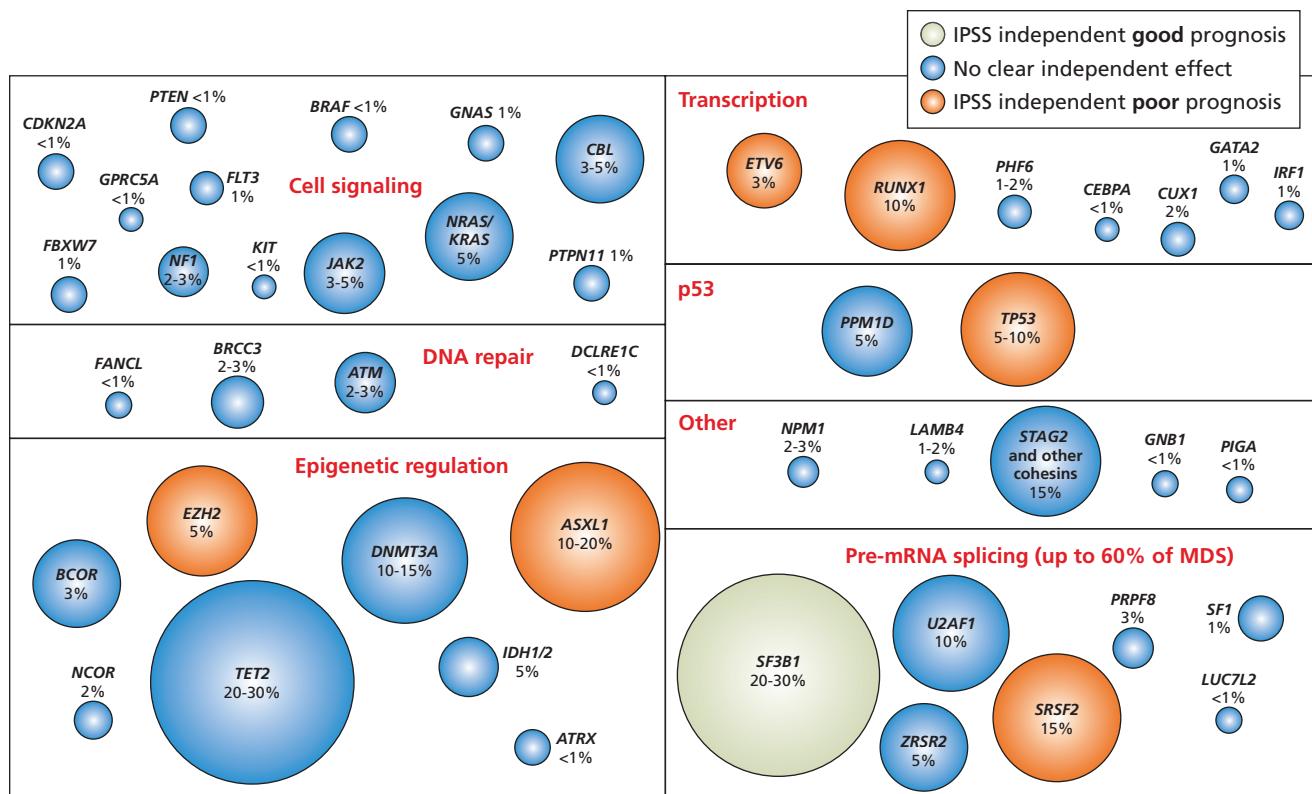


Figure 19-9 Recurrent somatic mutations in MDS, including approximate frequency of the most common recurrent somatic mutations in MDS and their prognostic significance. Some mutations influence the phenotype and are therefore more common in specific subtypes of MDS; for instance, *SF3B1* mutations are found in up to 80% of patients with MDS-RS, and *SRSF2* mutations are more common in MPN/MDS overlap syndromes such as chronic myelomonocytic leukemia. Mutation frequencies and their prognostic significance are derived from Bejar R et al, *N Engl J Med.* 2011;364:2496–2506; Haferlach T et al, *Leukemia.* 2014;28:241–247; and Papaemmanuil E et al, *Blood.* 2013;122:3616–3627. The negative prognostic impact with *SRSF2* mutations is from Thol F et al, *Blood.* 2012;119:3578–3584.

translocations involving the breakpoint at 11q23; the latency period between exposure and MDS/AML development is typically 1 to 3 years. These 11q23 translocations lead to transcription of a fusion protein involving the mixed-lineage leukemia (*MLL*) gene. Translocations and inversions of 3q21/3q26 can arise after etoposide treatment and involve rearrangement of the *MDS1-EVI1 (MECOM)* genes; such patients often have a normal or elevated platelet count at the time of diagnosis and have a grim prognosis.

KEY POINTS

- One-half of patients with *de novo* MDS and most patients with secondary, therapy-related MDS have a clonal cytogenetic abnormality.
- 5q– syndrome has a relatively benign prognosis, but not all patients with del(5q) have 5q– syndrome. Deletion of

RPS14, a gene on chromosome 5q that encodes a ribosomal subunit, contributes to the erythropoietic defect in del(5q) MDS and links del(5q) MDS to DBA, which is due to heterozygous germ-line mutations in genes, such as *RPS19*-encoding ribosomal proteins.

- Patients with t-MDS who have been exposed to alkylating agents or ionizing radiation usually have abnormalities of chromosomes 5 and 7, whereas those who have been exposed to epipodophyllotoxins usually have abnormalities of chromosome 11q23.
- More than 40 genes are known to harbor somatic mutations in patients with MDS. Those with IPSS-independent prognostic value include *EZH2*, *TP53*, *RUNX1*, *ASXL1*, and *ETV6*.

Cell biology

A major challenge in unraveling the complex pathogenesis of MDS is distinguishing primary events from secondary effects of specific initiating mutations within HSCs

and progenitor cells or the marrow microenvironment. As described above, MDS arises from DNA mutation-driven clonal expansion of multipotent or pluripotent HSCs or progenitor cells. Most studies of adults with MDS have shown that ineffective hematopoiesis, as opposed to the lack of hematopoietic activity that characterizes AA, is the major factor contributing to pancytopenia in MDS. Abnormal responses to cytokine growth factors, impaired cell survival, and defects in the bone marrow microenvironment are all implicated in the pathogenesis of MDS.

Analysis of X-linked polymorphisms and newer molecular techniques indicate that the malignant clone in MDS includes both CD34⁺ cells and more differentiated myeloid, erythroid, and megakaryocytic cells. B cells are sometimes part of the clonal process, but T cells are rarely involved, although distinct T-cell clones akin to those seen in large granular lymphocyte disorders may be detected in association with MDS. Cell culture studies with primary MDS samples have shown reduced growth of multilineage colony-forming unit (CFU)-granulocyte-erythroid-monocyte-megakaryocyte progenitors and of lineage-restricted burst-forming unit-erythroid, CFU-erythroid, CFU-granulocyte-macrophage, and CFU-megakaryocyte progenitors. These abnormalities in the progenitor compartment likely contribute to the development of peripheral blood cytopenias.

Experimental evidence also implicates inhibitory cytokines and increased intramedullary apoptosis as contributors to ineffective hematopoiesis in early MDS. Death receptor ligand binding may contribute to excessive apoptosis of hematopoietic precursors, resulting in ineffective hematopoiesis. For example, in several studies, bone marrow cells from patients with MDS demonstrated increased expression of Fas and Fas ligand, TGF β family members and their receptors, or of TNF α and its receptors. In marrow cultures, strategies that block TNF α -mediated signals or TGF β family members, such as the use of anti-TNF α antibodies or activin IIA/B receptor ligand traps that block erythropoiesis-inhibiting growth and differentiation factor 11 (GDF11), significantly increase the numbers of hematopoietic colonies compared with untreated cells. Increased apoptosis has been identified in both mature cells and immature CD34⁺ cells from patients with lower-risk MDS, compared with healthy controls and patients with higher-risk MDS or *de novo* AML. In patients with higher-risk MDS or AML, cell survival signals dominate.

Several studies have suggested that the bone marrow microenvironment is abnormal in MDS. The growth of stromal progenitors is defective, with reduced colony growth and failure of cultures to grow to confluence. Furthermore,

stromal support of the growth and maturation of normal hematopoietic progenitors also is impaired, consistent with a functional defect. Stromal cells may play an important role in the development and maintenance of abnormal signaling networks mediated by TNF α , Fas, and other soluble factors.

KEY POINTS

- MDS is clonal disorder that arises in hematopoietic stem and progenitor cells and affects the entire myeloid compartment. The heterogeneous nature of MDS and the advanced age at disease onset infer the existence of multiple cooperating genetic lesions.
- Whole genome sequencing shows that most patients with MDS have somatic nonsense or missense mutations.
- Both the "soil" (microenvironment) and the "seed" (hematopoietic progenitor cells) may be abnormal in MDS, contributing to failed hematopoiesis.
- Abnormal responses to cytokine growth factors, impaired hematopoietic progenitor cell survival and excessive intramedullary apoptosis, and defects in the marrow microenvironment have all been implicated in the pathogenesis of MDS.

Treatment of MDS

With the exception of allogeneic HSCT, no therapeutic options in MDS have demonstrated curative potential. However, three medications have specific U.S. FDA approval for MDS-related indications (azacitidine, decitabine, and lenalidomide), and these drugs offer benefit to a subset of patients. Advanced age, the presence of comorbidities, and a lack of a suitable donor limit the availability of allogeneic HSCT, but use of reduced-intensity conditioning approaches and alternative stem cell sources (eg, umbilical cord blood and mismatched donors, including haploidentical donors) are expanding the roster of potentially eligible patients. Therefore, patients with MDS who are potential candidates for transplantation should be evaluated early in the disease course by a physician with expertise in stem cell transplantation. In many centers, reduced-intensity stem cell transplantation is now routinely performed for patients in their 60s and 70s.

Goals of MDS therapy for an individual patient depend in part on the stage of disease and include symptom control, reduction of transfusion needs, delay of disease progression, and extension of survival. Prognostic systems such as the IPSS-R, supplemented by molecular testing, allow clinicians to incorporate clinicopathological risk factors for death and disease progression into therapeutic decisions.

Supportive care: transfusions and iron chelation

Despite the availability of several active treatments for MDS, transfusion support remains a mainstay of therapy for many patients. Patients receiving red blood cell (RBC) transfusions at least once every 8 weeks have a poorer survival than those who do not require regular transfusions, probably because a need for transfusions is a marker of more advanced hematopoietic failure and higher-risk disease. In a number of studies, lower-risk patients with MDS who have a ferritin >1,000 ng/mL have been shown to experience poorer survival than lower-risk MDS patients with a ferritin ≤1,000 ng/mL, suggesting that transfusion-related iron overload also might be a contributing factor to poorer outcomes in transfusion-dependent patients. In light of this, RBC transfusions should be minimized and utilized only as necessary for symptomatic anemia or to maintain a safe hemoglobin of 7 to 8 g/dL. When utilized, RBC transfusions should be leukocyte-reduced to avoid risk of transfusion-associated GVHD.

Because the correlation between serum ferritin and iron burden is relatively poor and patients receiving transfusions develop iron overload at different rates, newer techniques for noninvasively measuring hepatic iron concentration, such as quantitative ($R2^*/T2^*$) magnetic resonance imaging, may be useful in determining which patients are the best candidates for iron chelation. Cardiac $T2^*$ magnetic resonance imaging results may also be clinically helpful in determining patients' risk from iron overload, but $T2^*$ signals in the heart are rarely abnormal until patients have received at least 80 to 100 units of blood.

Consideration should be given to initiation of iron chelation therapy with parenteral deferoxamine or oral desferrioxamine in low-risk MDS patients who have a reasonable life expectancy, are red blood cell transfusion dependent, and have evidence of tissue iron overload. No controlled prospective data, however, support a survival benefit from iron chelation in MDS, and such therapy is costly and can have adverse effects. In elderly patients with MDS, a dose of desferrioxamine high enough to cause a negative iron balance (ie, at least 20 to 30 mg/kg/d) often results in elevated creatinine or intolerable gastrointestinal symptoms. Desferrioxamine and deferoxamine should be avoided in patients with creatinine clearance less than 40 mL/min. Deferiprone (L1) is widely used for chelation therapy in thalassemia, but a risk of agranulocytosis limits its use in MDS. Platelet transfusions also may be necessary in some patients with MDS who have bleeding episodes, but the development of alloimmunization is problematic.

Hematopoietic growth factors

Hematopoietic growth factors are an integral part of the treatment of MDS, despite the lack of a specific FDA-approved

indication for any of the available agents. Erythropoiesis-stimulating agents (ESAs) in particular may reduce transfusion requirements by improving hemoglobin levels, and these agents are generally well tolerated.

Studies with recombinant ESAs (epoetin and darbepoetin) demonstrated erythroid response rates in the range of 20% to 40%. The combination of ESA and G-CSF may be more effective in improving anemia than treatment with ESA alone, especially in patients with MDS-RS. No prospective studies have shown an alteration in survival with ESAs in MDS, although several retrospective studies suggest that ESAs may improve life expectancy and there is no increase in AML progression with ESA use.

An 8- to 12-week trial of an ESA at standard dosing schedules is appropriate for anemic patients with serum erythropoietin levels <200 to 500 U/L. Patients with serum erythropoietin levels >500 U/L respond only rarely to ESA therapy, and patients who have heavy transfusion needs are less likely to respond than those who do not require transfusions.

Both G-CSF (filgrastim, tbo-filgrastim) and granulocyte-macrophage colony-stimulating factor (sargramostim, molgramostim) have been evaluated in patients with MDS and increase the neutrophil count in up to 60% to 90% of patients, which may help some patients who have recurrent infections. Nonetheless, currently this practice is usually discouraged overall. Concerns regarding use of G-CSF and risk of leukemic transformation were addressed in a randomized controlled trial of 102 patients with high-risk MDS who were treated with either G-CSF or supportive care. No differences in frequency or time to progression to AML were seen between the two groups overall, but survival was shorter in patients with 5% to 19% blasts who received G-CSF. Pegfilgrastim has been associated with splenic rupture and leukemoid reactions in MDS and, if used, should be administered only with caution and started at low doses (eg, 1 to 3 mg, rather than the standard 6-mg vial). However, there has been no proven benefit to the use of pegfilgrastim in MDS.

Thrombopoietin (TPO)-receptor agonists approved for use in immune thrombocytopenia, romiplostim, and eltrombopag have been evaluated in clinical trials for patients with lower-risk MDS and can improve the platelet count in many patients and reduce bleeding events. Patients who are not heavily platelet transfusion dependent and who have an endogenous TPO level <500 pg/mL are most likely to benefit. However, some patients experience an increase in blood or marrow blast proportion during romiplostim or eltrombopag therapy, which may be because some myeloblasts have functional TPO receptors. In one placebo-controlled study of romiplostim monotherapy, progression to AML was observed in 6% of patients treated with romiplostim,

compared with 2.4% with placebo, with the majority of progressions seen among patients who already had excess blasts before treatment. When the drug is withdrawn, the blast percentage usually decreases and the survival has not been shown to be impacted. When romiplostim was used in pilot studies in combination with azacitidine, decitabine, or lenalidomide, however, an increased rate of progression to AML was not observed. Another concern with TPO agonists is the possibility of development of marrow fibrosis with long-term use, because mice engineered to over-express TPO develop a myelofibrosis-like picture, but the clinical relevance of this is unclear and, to date, fibrosis in TPO agonist-treated patients with MDS has been rare. Rebound thrombocytopenia can occur with discontinuation of TPO agonists. Thrombocytopenic patients who have bleeding from mucosal surfaces (eg, urinary bladder or gut) may benefit from topical therapy or careful use of the anti-fibrinolytic agent epsilon aminocaproic acid.

KEY POINTS

- Transfusion support with leukocyte-depleted blood products is an integral part of supportive care for most patients with MDS. Iron chelation may become necessary in carefully selected low-risk patients who are receiving regular red cell transfusions.
- Data are insufficient to determine whether treating MDS patients with hematopoietic growth factors alters disease progression or survival.
- ESAs lead to a red blood cell response in ~20% to 40% of patients; adding G-CSF to ESAs can lead to red blood cell response in ~40% of patients, and responses to combined therapy may be more common among patients with MDS-RS.
- ESAs are less effective in patients with high pretreatment serum erythropoietin levels ($\geq 500 \text{ U/L}$).
- TPO receptor agonists (thrombopoiesis-stimulating agents) can raise the platelet count in some patients with MDS and decrease platelet transfusions and clinically significant bleeding events, but they have been associated with increased blast proportion in some cases and are not FDA approved for MDS.

for regulating gene transcription. DNA methyltransferase 1 (DNMT1) is the enzyme responsible for maintenance of cytidine methylation patterns, and the aza-substituted cytosine nucleoside analogs azacitidine and decitabine can inhibit DNMT1 by incorporating into RNA or DNA and irreversibly binding to this enzyme, resulting in generalized hypomethylation of DNA and reversal of gene silencing. Although these so-called epigenetic changes occur *in vitro* in cells exposed to DNMT1 inhibitors, it is not clear whether these epigenetic effects are responsible for the clinical activity of azacitidine or decitabine in MDS or whether other biologic effects (eg, DNA damage) also play a role.

Azacitidine is the first and, as of this writing, only medication that has been shown in a randomized trial to improve survival in higher-risk MDS patients. In a multicenter trial (AZA-001), 358 patients with IPSS intermediate-2 or high-risk MDS were randomized to receive either azacitidine 75 mg/m^2 subcutaneously for 7 consecutive days every 28 days or conventional care (ie, best supportive care, either alone or with low-dose cytarabine or AML-like induction chemotherapy using infusional cytarabine and an anthracycline). The median survival time was 24 months in patients receiving azacitidine vs 15 months in patients receiving conventional care. Although the complete response rate in the azacitidine-treated group was a modest 17%, subsequent analysis demonstrated that a complete response was not necessary for patients to achieve a survival benefit; however, it is unclear whether stable disease alone or minor hematologic improvements are beneficial. Azacitidine is approved for intravenous administration and subcutaneous dosing. Intravenous administration avoids injection site reactions, but requires either central or peripheral venous access.

Decitabine is also active in MDS, but a European multicenter study designed to show a survival benefit with decitabine in MDS was negative. The overall survival of the control arm in that study (8 months) suggests that a different population was enrolled compared to AZA-001.

Clinical response to hypomethylating agents may be delayed, and an adequate therapeutic trial of either agent requires at least four to six treatment cycles. Although the initial FDA approval of decitabine was for a regimen of 15 mg/m^2 administered every 8 hours for 9 doses intravenously (in a hospital-based setting), the most commonly used regimen in clinical practice is 20 mg/m^2 intravenously once daily for 5 consecutive days, repeated every 4 to 6 weeks. In a multicenter study of this 5-day decitabine regimen, 17% of patients achieved a complete response, 15% achieved a marrow response, and 18% experienced hematologic improvement, similar to the response rates observed with azacitidine therapy.

Hypomethylating agents (DNA methyltransferase inhibitors)

Cytidine residues in mammalian DNA can be methylated, and DNA methylation is a dynamic process that affects transcription rates. Methylated cytidine residues cluster in so-called cytosine-phosphate-guanine islands, which are located near the promoter regions of many genes. When these regions are hypermethylated, expression of nearby genes is silenced, and this represents a mechanism

The most common adverse events associated with both hypomethylating agents are neutropenia and thrombocytopenia, which often improve over time with continued treatment as the MDS clones are suppressed and normal hematopoiesis recovers. The optimal maintenance dosing once patients achieve a response is unknown, but some maintenance therapy appears to be required to retain responses. Thus far, no therapy has been demonstrated to improve survival for patients with lower-risk MDS, though DNA hypomethylating agents can improve peripheral counts and reduce transfusion needs in a minority of such patients.

Given the frequency of mutations in pathways that alter DNA methylation in MDS, it is reasonable to hypothesize that such mutations might serve as biomarkers for therapeutic response to hypomethylating agents. Indeed, in both a French study and in a trial run by the defunct Bone Marrow Failure Consortium, the presence of mutations in *TET2* or *DNMT3A* predicted a modestly higher likelihood of response to azacitidine therapy. The response rate was high enough in the wild-type group, however, that this mutation signature cannot be used to select therapy in the clinic.

Once hypomethylating agents fail the patient, the prognosis is grim, with a median survival <6 months. Switching from one failed hypomethylating agent to the other agent or adding additional agents such as lenalidomide is usually not helpful. Patients who fail hypomethylating agents should be referred for HSCT or enrolled in clinical trials whenever feasible. Responses are seen in some patients with low-dose cytarabine or clofarabine.

Immunomodulatory drugs

The drug thalidomide has multiple biological effects, including alteration of immune cell subsets, inhibition of TNF α and other cytokines, and inhibition of neoangiogenesis in the marrow. These effects are mediated by modulation of the activity of an E3 ubiquitin ligase complex that includes the protein cereblon. When thalidomide was used for MDS in the 1990s, responses were seen in ~20% of patients, but the drug was difficult to tolerate (especially for elderly patients) due to sedation, constipation, peripheral neuropathy, and other adverse events.

Lenalidomide was then generated by chemical modification of thalidomide, and has an improved safety profile without the neurologic toxicity seen with thalidomide. Lenalidomide has more potent immunomodulatory, anti-TNF α , and anti-vascular endothelial growth factor effects than thalidomide. Its primary mechanism is via cereblon-mediated alteration in the degradation rate of casein kinase 1, a serine-threonine kinase that is encoded on chromosome 5q and modulates Wnt/ β -catenin signaling.

After phase 1 testing suggested a high response rate in del(5q) MDS, lenalidomide was tested in a phase 2 trial in patients with IPSS low-risk or intermediate-1-risk disease who were red blood cell transfusion dependent and had a deletion of chromosome 5q31, either alone or in association with other chromosomal abnormalities. Of 148 patients enrolled in this phase 2 study, 67% achieved transfusion independence, with a median time to response of 4.6 weeks. The median increase in hemoglobin was 5.4 g/dL and the median duration of response was >2 years. A major cytogenetic response (ie, elimination of the del(5q) clonal abnormality) occurred in 44% of patients. The major adverse effect was myelosuppression, with grade 3 to 4 neutropenia and thrombocytopenia seen in up to 55% of patients; treatment-emergent cytopenias are associated with a moderately higher likelihood of response. These results led to the approval of lenalidomide by the FDA in 2005 for patients with del(5q) with IPSS low-risk or intermediate-1-risk disease who are red blood cell transfusion dependent.

A second phase 2 trial of lenalidomide was conducted in patients with the same eligibility who did not have del(5q). In this patient population, responses were less frequent and of shorter duration compared with those in patients with del(5q); 26% of patients became red blood cell transfusion independent, with a median response duration of 41 weeks. A third trial was conducted comparing a starting dose of 5 mg daily to 10 mg for 21 out of 28 days in patients with del(5q) MDS, because many patients starting at the 10-mg dose require dose reduction due to treatment-emergent cytopenias. Complete response rates and cytogenetic response rates were superior in the 10-mg arm. A placebo-controlled trial did not show an increase in disease progression with lenalidomide use.

Lenalidomide at high doses (>10 mg/d) has some clinical activity in patients with high-risk disease (eg, excess blasts or complex karyotype) or AML, but is not FDA approved for these indications. Patients with a low platelet count are much less likely to achieve benefit from lenalidomide than those with a platelet count >50 \times 10 9 /L.

Immunotherapy

An autoreactive T-cell-mediated process suppressing hematopoiesis may contribute to pancytopenia in some patients with MDS. Several studies have demonstrated that treatment approaches analogous to IST of AA may be beneficial in MDS. Therapy with ATG and CSA (as described in the AA section) benefits some patients with lower-risk disease (<10% blasts), especially those who are <60 years of age, lack transfusion dependence, show marrow hypocellularity, and have either a normal karyotype or

trisomy 8. Selection of patients most likely to respond to ATG or cyclosporine therapy remains challenging

KEY POINTS

- Azacitidine has been demonstrated to improve survival by a median of 9 months in patients with higher-risk MDS. Decitabine, another hypomethylating cytosine analog, also produces responses in MDS and delays AML progression. Both drugs are approved by the FDA for the treatment of MDS.
- Azacitidine and decitabine induce DNA hypomethylation through the inhibition of DNMT1, but it is not clear whether this mechanism is responsible for the clinical effects.
- Lenalidomide led to transfusion independence in 67% of lower-risk MDS patients with deletions of chromosome 5q, and some patients also achieved a cytogenetic remission. Lenalidomide also has some effectiveness in anemic patients with lower-risk MDS who lack deletion of chromosome 5q, but the response rate is only 20% to 25%, and responses are not durable.
- Lenalidomide's mechanism of action in MDS is via binding to cereblon and modulation of ubiquitination of casein kinase 1 and alteration of casein kinase's clearance rate. Casein kinase is encoded on chromosome 5q.
- Some patients with hypocellular MDS respond to ATG or cyclosporine/tacrolimus immunotherapy, but selecting the most appropriate patients for this therapy remains challenging. Younger patients and those with lower-risk disease (ie, those who are not yet transfusion dependent or have required transfusions for only a short time) with normal karyotype or trisomy 8 seem most likely to benefit.

Allogeneic HSCT

Even though allogeneic HSCT is the only potentially curative therapy in MDS, <10% of patients with MDS currently undergo HSCT due to older age, comorbid conditions, lack of a suitable donor, high cost, patient concern over the risk of transplantation, and failure of clinicians to refer patients who might be transplant candidates to transplant centers. Younger patients (ie, <40 years) without excess blasts at the time of transplant may have a long-term disease-free survival rate exceeding 50% after an HLA-matched HSCT. Patients with high IPSS score or treatment-resistant disease have survival rates <30% after HSCT. Patients with a complex monosomal karyotype, defined as two or more autosomal monosomies or one monosomy plus additional structural chromosomal abnormalities, are at particularly high risk for poor outcome. When patients with complex karyotype also have a *TP53* mutation, the long-term disease-free survival even with HSCT is <10%.

Allogeneic HSCT should be considered for patients with higher-risk MDS who have an adequately HLA-matched donor and a good performance status. The use of reduced-intensity conditioning regimens may permit allogeneic HSCT in older individuals up to the age of 75.

Given the risks of allogeneic HSCT, defining the optimal time to refer patients for transplantation is an important consideration. One analysis indicated that performing transplantation in patients with lower-risk disease (ie, IPSS low and intermediate-1 risk) only at the time of progression of disease resulted in greater life expectancy than when HSCT was performed earlier in the disease course. In contrast, patients with higher-risk disease (IPSS intermediate-2 and high risk) benefited from HSCT shortly after diagnosis. Unfortunately, disease relapse occurs in the majority of high-risk patients after HSCT and thus represents a continuing challenge. Prognostic models to assess HSCT risk have been proposed that stratify by many of these factors. Strategies to reduce relapse rates are being studied and include pre- and posttransplantation interventions with novel therapies. No clear benefit has been shown for the administration of one or more courses of cytotoxic chemotherapy or hypomethylating agent therapy before HSCT, although pretransplantation therapy may be useful to reduce the burden of marrow blasts to <10% before the HSCT. Recent publications have also shown that genetic profiling pre-HSCT can predict clinical outcomes post-HSCT as well as inform selection of conditioning. *TP53* mutations were associated with shorter survival after transplant, as were *RAS* pathway mutations. There was no benefit to myeloablative regimens over reduced-intensity regimens in patients with *TP53* mutations.

HSCT is the treatment of choice for children and young adults with MDS. It is imperative to perform a diepoxybutane test to exclude FA before performing a transplantation in a child or young adult with apparently *de novo* MDS, because patients with FA suffer severe toxicity with conventional conditioning regimens and also require close monitoring for nonhematologic tumors after transplantation. Although most patients with FA have dysmorphic features such as short stature and radial ray anomalies, many do not. A bone marrow examination and cytogenetic testing should be performed on any related donor when the recipient is a child or young adult with bone marrow monosomy 7, because there have been instances in which an unsuspected clonal disorder has been detected in the prospective donor. Particular care must be exercised in determining the proper conditioning regimen and best time to perform HSCT in infants and young children because of the toxic effects of radiation on the developing central nervous system and because of differences in drug metabolism compared with older children and adults.

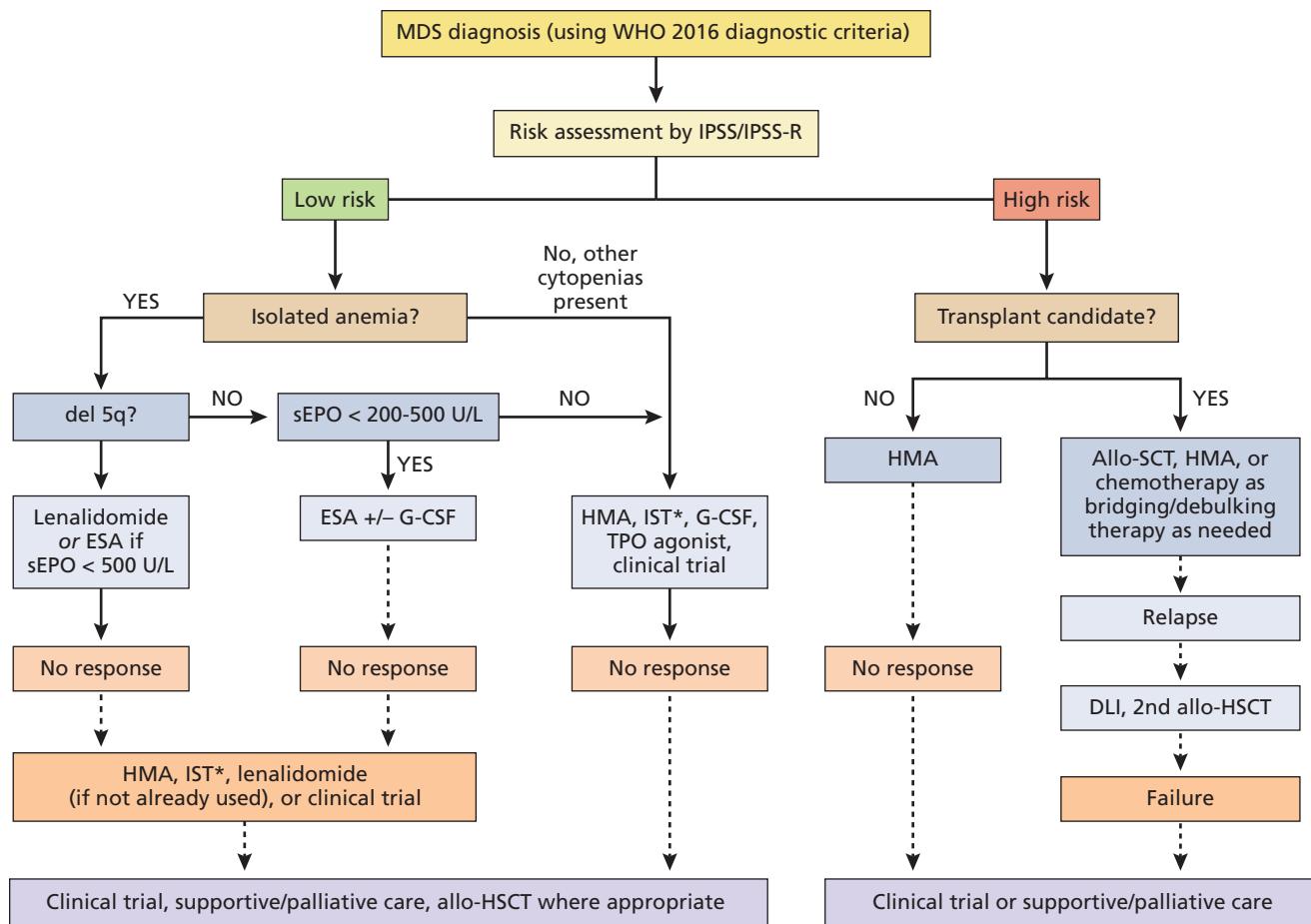


Figure 19-10 A general approach to MDS therapy, as described in the accompanying text. All patients should receive supportive care. Low-intensity therapies are most suited for lower-risk MDS, whereas patients with higher-risk disease should move to allogeneic stem cell transplant, if feasible, or otherwise be considered for azacitidine or decitabine therapy. allo-SCT, stem cell transplantation; DLI, donor lymphocyte infusion; HMA, hypomethylating agent (azacitidine or decitabine); sEPO, serum erythropoietin level. Based on National Comprehensive Cancer Network guidelines; see <http://www.nccn.org>. *Candidates for IST may include: (1) patients who are age 60 years or younger with less than or equal to 5% marrow blasts; (2) patients who have hypocellular marrows; (3) patients with HLA-DR15 positivity; (4) patients with PNH clone positivity; or (5) patients with T-cell clones.

KEY POINTS

- Allogeneic HSCT remains the only routinely curative approach in MDS and is an important consideration if the patient is young and otherwise healthy and has an HLA-identical sibling or a closely matched unrelated donor. Cure rates overall are ~30% to 40%.
- Reduced-intensity (nonmyeloablative) conditioning regimens are associated with a lower transplantation-related mortality but higher relapse rate in MDS; overall survival is similar with reduced-intensity and conventional myeloablative conditioning. Reduced-intensity conditioning regimens may permit HSCT to be performed in older and sicker patients who would not tolerate myeloablative conditioning.

- Transplantation at the time of progression for patients with lower-risk disease, and as soon as feasible after the time of diagnosis for patients with higher-risk disease, yields the greatest life expectancy.
- HSCT is the treatment of choice for pediatric MDS; however, donors and recipients must be screened carefully to exclude familial disorders such as FA that would alter the management.

General therapeutic approach

An approach to MDS therapy is outlined in Figure 19-10. All patients should receive supportive care with transfusions and antimicrobial agents as needed. Iron chelation

therapy can be considered for selected RBC transfusion-requiring, lower-risk patients with an expected long life expectancy and evidence of transfusional hemosiderosis.

For lower-risk patients (ie, those without excess blasts or an adverse karyotype) in whom the clinical picture is dominated by anemia, the initial therapeutic choice depends on the karyotype and the serum erythropoietin (EPO) level. For patients with del(5q), lenalidomide is an appropriate first choice and is FDA approved for this indication. For patients without del(5q) but with serum EPO <200 to 500 U/L, ESAs such as epoetin or darbepoetin are recommended. Iron stores should be monitored with ESA therapy and repleted if needed.

The most appropriate therapy for lower-risk patients with either anemia with serum EPO >500 U/L and without del(5q), pancytopenia, or a clinical picture dominated by individual cytopenias other than anemia (ie., neutropenia or thrombocytopenia) is unclear. Hypomethylating agents can be beneficial in some patients with lower-risk disease, though their effect on survival in this group is unclear. Patients with isolated thrombocytopenia may overlap with immune thrombocytopenia and may benefit from corticosteroids, romiplostim, intravenous gamma globulin, or other immune thrombocytopenia-directed therapies. IST, lenalidomide, supportive care alone, or HSCT are all reasonable choices in the other patient groups, depending on patient-specific factors. Many of the patients in these groups do not truly have “lower-risk” disease—for instance, the population with pancytopenia is enriched for those with EZH2 mutations, which confers increased risk—and, in the future, molecular profiling may help assign them to a higher-risk group, likely resulting in increased therapy with hypomethylating agents or other potentially disease-modifying approaches.

For higher-risk patients, the treatment approach differs depending on whether the patient is a transplant candidate. Higher-risk patients who are HSCT candidates should proceed with definitive HSCT therapy as soon as feasible. HSCT may be preceded by a few treatment cycles of a hypomethylating agent as a “bridging” therapy to try to cytoreduce or at least keep the disease stable until a donor is identified, insurance approval is obtained, and pretransplant screening tests are completed. Patients who are not HSCT candidates can be treated with a hypomethylating agent; some investigators prefer azacitidine over decitabine because of the demonstrated survival advantage in this setting.

Once initial therapy fails, no optimal second-line therapy is defined, and the choice depends on clinical circumstances. Supportive care is the default, and clinical trial enrollment is always appropriate, if available.

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The online version of this chapter contains an educational multimedia component on acute myeloid leukemia.

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Dr. Stein has served on advisory boards for Celgene, Agios, Novartis, Astellas, Bayer, and Pfizer. Dr. Shukla declares no competing financial interest. Dr. Altman has sat on advisory boards for AbbVie, Agios, Astellas, Daiichi Sankyo, Novartis, and Theradex and a data monitoring committee for Glycomimetics.

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Acute myeloid leukemia

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Definition and epidemiology

Acute myeloid leukemia (AML) is a heterogeneous clonal hematopoietic progenitor or stem cell malignancy in which immature hematopoietic cells proliferate and accumulate in bone marrow, peripheral blood, and other tissues (see video in online edition). This process results in inhibition of normal hematopoiesis, characterized by neutropenia, anemia, thrombocytopenia, and the clinical features of bone marrow failure. AML accounts for 90% of all acute leukemias in adults, with an estimated 19,520 new cases and 10,670 deaths expected in the United States in 2017. The annual incidence is approximately 3.5 per 100,000 and increases with age, with approximately a 10-fold increased risk between ages 30 (1 case per 100,000) and 65 years (1 case per 10,000). The median age at diagnosis is approximately 68 years, with approximately 6% of patients younger than 20 years and 34% of patients 75 years or older. Overall survival in adults remains poor, with <50% 5-year survival in patients younger than 45 years that continues to fall to <10% in patients older than 60 years at diagnosis. In children, overall survival has improved to approximately 60%.

Most cases of AML have no apparent cause. Some patients may have the emergence of abnormal myeloid clones in the bone marrow, termed clonal hematopoiesis, years before diagnosis. The most common known risk factor is previous exposure to radiation or chemotherapy, particularly topoisomerase II inhibitors and alkylating agents, which result in therapy-related AML (t-AML) and accounts for ~10% to 20% of all AML cases. The incidence of AML arising after exposure to alkylating agents or radiation therapy increases with age, typically has a 5- to 10-year latency period, and frequently is associated with an antecedent therapy-related myeloid neoplasm (t-MN), such as myelodysplastic syndrome (MDS) and unbalanced loss of genetic material involving chromosomes 5 or 7 and/or a mutation in TP53. T-AML associated with exposure to topoisomerase II inhibitors is less common, encompasses 20% to 30% of patients with t-AML, has a shorter latency period of 1 to 5 years, is less often preceded by a myelodysplastic phase, and may be associated with balanced recurrent chromosomal translocations involving 11q23 (MLL gene) or 21q22 (RUNX1). Other environmental risk factors include exposure to benzene and ionizing radiation. Familial AML, caused by germ line mutations in CEBPA, DDX41, and other genes, occurs in a small subset of patients. Familial platelet disorder with propensity to myeloid malignancies results from

germ line mutations in the RUNX1 gene. Patients with inherited bone marrow failure syndromes (eg, Fanconi anemia, Shwachman-Diamond syndrome, and severe congenital neutropenia), genetic disorders (eg, Down syndrome), and MDS and myeloproliferative neoplasms are also at increased risk of developing AML. The recognition of familial AML and inherited bone marrow failure syndromes are important when deciding on a donor source for patients who will receive an allogeneic bone marrow transplant. In addition, patients with a first-degree relative with a history of a myeloid neoplasm should be considered for referral to clinical genetics and germ line testing for a heritable cause of their AML.

Clinical manifestations

Patients with AML generally present with signs and symptoms related to infiltration of the bone marrow and other organs with leukemic blasts and the resultant symptoms of anemia and thrombocytopenia. Symptoms include pallor, fatigue, bone pain, hepatosplenomegaly, fever, bruising, and bleeding. Tissue infiltration of the skin, gingiva, and central nervous system (CNS) is more common with monocytic subtypes. CD56 expression, in addition to monocytic subtypes, increases extramedullary risk at presentation. Patients with leukocytosis and leukemic blasts >50,000/ μ L are at increased risk of pulmonary and CNS complications from leukostasis. Pathologically, this process shows a combination of microinfarction and hemorrhage.

AML may be associated with a variety of laboratory derangements in addition to abnormal blood counts. Coagulation abnormalities are particularly common and severe in patients with acute promyelocytic leukemia (APL), but these abnormalities may be present in all subtypes. Metabolic abnormalities related to tumor lysis syndrome, including hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia, also may be present. Patients with monocytic leukemia may exhibit severe hypokalemia.

Diagnostic workup

The diagnostic evaluation for patients with suspected AML consists of studies to confirm the diagnosis, establish the AML subtype (see below), and stratify risk. All patients should undergo a bone marrow aspiration and biopsy to clearly establish the presence of >20% myeloblasts on an aspirate smear and an immunophenotyping by flow cytometry to confirm the blasts are of myeloid lineage. In addition, traditional cytogenetics, fluorescence in situ hybridization (FISH) (if there are inadequate cells in metaphase for cytogenetics) and molecular genetics for mutant genes as published in national consensus guidelines (*FLT3*, *NPM1*, *CEBPA*, *ASXL1*, *RUNX1*, *TP53*) should also be undertaken.

Subtype classification

In the 1970s, AML was subclassified according to the French-American-British (FAB) classification system using morphologic and cytochemical criteria to define eight major AML subtypes (M0 to M7) on the basis of greater than or equal to 30% blasts, lineage commitment, and the degree of blast cell differentiation. The FAB system has been replaced by the World Health Organization (WHO) classification, which was developed to incorporate epidemiology, clinical features, biology, immunophenotype, and genetics into the diagnostic criteria. WHO has identified a number of genetically defined subgroups of AML (Table 20-1).

AML is defined as greater than or equal to 20% myeloblasts, monoblasts, or promonocytes, erythroblasts, or megakaryoblasts in the peripheral blood or bone marrow,

Table 20-1 World Health Organization 2016 classification of acute myeloid leukemia (AML) and related myeloid neoplasms

Acute myeloid leukemia and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1); <i>RUNX1-RUNX1T1</i>
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>
APL with <i>PML-RARA</i>
AML with t(9;11)(p21.3;q23.3); <i>MLLT3-KMT2A</i>
AML with t(6;9)(p23;q34.1); <i>DEK-NUP214</i>
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); <i>GATA2</i> , <i>MECOM</i>
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); <i>RBM15-MKL1</i>
Provisional entity: AML with <i>BCR-ABL1</i>
AML with mutated <i>NPM1</i>
AML with biallelic mutations of <i>CEBPA</i>
Provisional entity: AML with mutated <i>RUNX1</i>
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia

except in patients with the following cytogenetic abnormalities and who are classified as having AML irrespective of blast count: t(8;21)(q22;q22), inv(16)(p13q22), t(16;16) (p13;q22), and t(15;17)(q22;q12). Immunophenotypic characterization using surface antigens remains important in AML and may include progenitor-associated antigens (eg, human leukocyte antigen-DR [HLA-DR], CD34, CD117) and myeloid antigens (eg, CD13, CD33). Complex composite immunophenotypes, including expression of lymphoid markers also may be present.

Pronostic factors

AML is a clinically and biologically heterogeneous disease. Adverse clinical prognostic features include advanced age at diagnosis, extramedullary disease (including CNS leukemia), disease related to previous chemotherapy or radiation treatment (t-AML), and the presence of an antecedent hematologic disorder (typically myelodysplastic syndrome [MDS] or myeloproliferative disorders). Patients older than 60 years, and especially those older than 75 years, have poor long-term survival because of disease- and host-related factors, medical comorbidities, and poor performance status.

Chromosomal (cytogenetic) and molecular abnormalities present in the leukemic myeloid blasts are the primary tools used in assigning prognosis for patients with newly diagnosed AML. Acquired, clonal chromosomal abnormalities, including balanced translocations, inversions, deletions, monosomies, and trisomies may be found in as many as 50% of patients with AML. The karyotype is considered complex when there are more than three abnormalities, which is found in 10% to 20% of patients, often in association with a *TP53* gene mutation or deletion. Cytogenetic findings remain an important prognostic tool and are classified into favorable, intermediate, and unfavorable risk groups. It is universally agreed that patients with the t(15;17) (q22;q12-21), found in acute promyelocytic leukemia (APL), have excellent outcomes. Balanced abnormalities of t(8;21)(q22;q22), inv(16)(p13.1 q22), and t(16;16)(p13.1;q22) involve the heterodimeric components of core-binding factor (CBF) and are associated with a relatively favorable prognosis. Complex karyotype, inv(3) (q21q26)/t(3;3)(q21;q26), and monosomal karyotype (at least two autosomal monosomies or one single-autosomal monosomy combined with at least one structural abnormality) are associated with particularly poor outcomes.

Molecular alterations also provide important prognostic information for many patients with AML, particularly those with a normal karyotype. (Figure 20-1). These patients form the largest cytogenetic subset of AML and, without further ability to classify them, most generally fall into an intermediate-risk group. Yet, these intermediate-risk pa-

tients have variable outcomes with conventional treatment strategies, which may be explained by the underlying molecular heterogeneity associated with their disease. For example, 20% to 25% of patients with AML have fms-like tyrosine kinase 3 (*FLT3*) length mutations (inclusive of internal tandem duplications [ITDs], insertions, and deletions), which are associated with an inferior prognosis. In addition, heterozygous mutations in exon 12 of the nucleophosmin member 1 (*NPM1*) gene have been found in 40% to 60% of AML patients with a normal karyotype, and mutated *NPM1*, in conjunction with wild-type *FLT3*, is associated with a favorable prognosis. Biallelic mutations of the CCAAT-enhancer-binding protein A gene (*CEBPA*), a gene encoding a myeloid transcription factor important for normal granulopoiesis, also appear to be associated with favorable clinical outcomes. Certain mutations, such as *IDH1/ IDH2* and *FLT3*, may have therapeutic implications because specific inhibitors of these mutant proteins are available.

Whereas evaluating for mutations in *NPM1*, *FLT3*, and *CEBPA* has become part of routine testing to aid in risk stratification for patients with AML associated with a normal karyotype, a host of other molecular alterations, including mutations in genes defining epigenetic pathways, such as *DNMT3A*, *IDH1*, *IDH2*, *TET2*, and others, have been described in many patients with AML. In addition, genetic profiling of patients with a normal karyotype has started to yield insights into distinct prognostic subgroups of patients with various co-occurring mutations. The Eastern Cooperative Oncology Group created genetic profiles of all of the patients treated under protocol E1900, a randomized trial of 90 mg/m² daily vs 45 mg/m² daily of daunorubicin with both trial arms receiving 7 days of infusional cytarabine. Analysis demonstrated that certain mutations, such as *NPM1* and *FLT3-ITD*, co-occur frequently, while others, such as *IDH* mutations and *TET2* mutations, appear to be mutually exclusive, leading to insights into pathways of leukemogenesis and hierarchies of clonal evolution. In addition, combining clinical outcomes with genetic profiling has started to define new groups of patients with a favorable prognosis. Finally, identification of these mutations has served as a platform for the development of novel therapeutic inhibitors of the mutated proteins and has led to ongoing efforts to develop clinical trials combining novel agents to target each genetic alteration. Because of the prognostic and therapeutic implications of certain genetic alterations, molecular genetic analysis for mutations with prognostic and therapeutic impact are imperative at the time of diagnosis. These mutations include *NPM1*, *FLT3*, *CEBPA*, *TP53*, *RUNX* and *ASXL1*, *IDH2*, and *IDH1*. The ideal sequencing platform depends on the gene of interest and the desired depth of analysis.

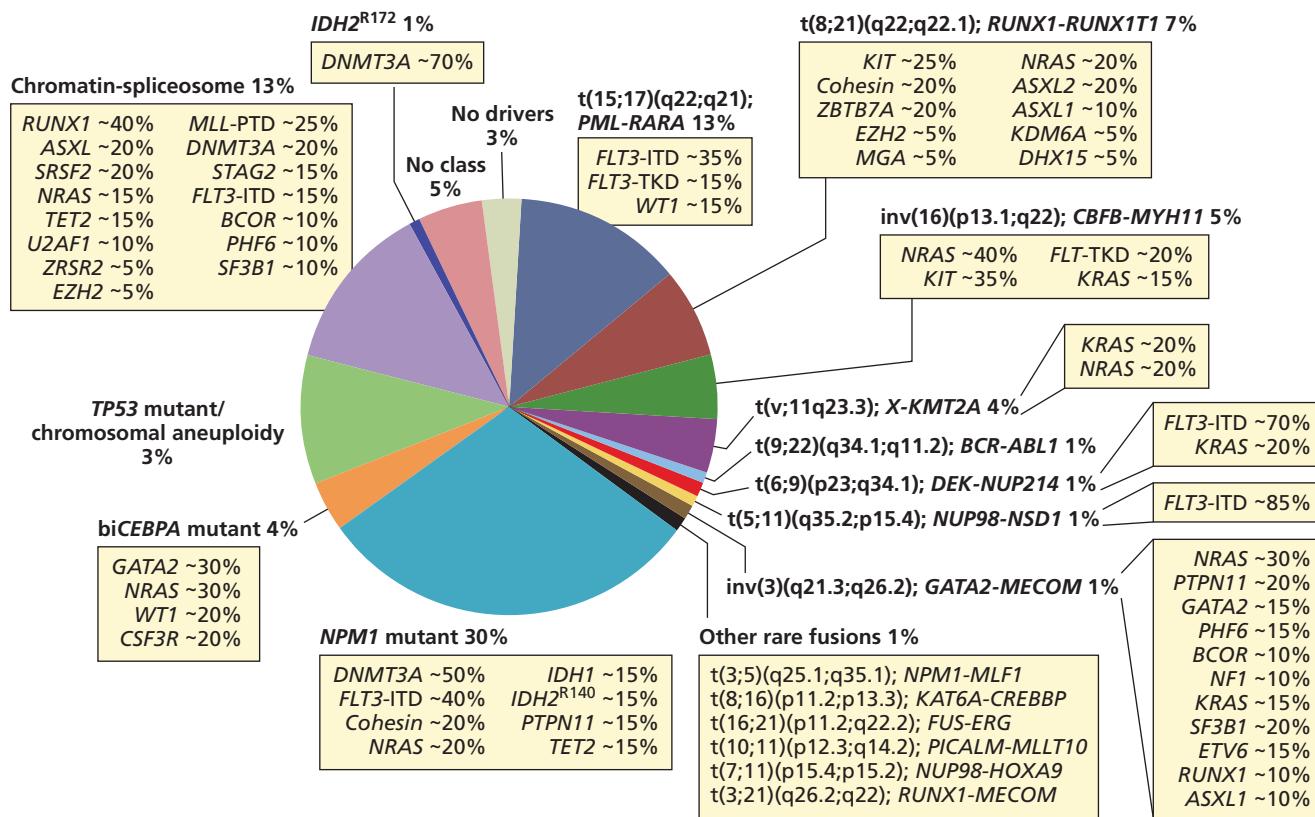


Figure 20-1 Major cytogenetic and molecular genetic subgroups of AML (and associated gene mutations). Redrawn from Döhner H, et al, *Blood*. 2017;129(4):424–447, with permission.

Recent efforts to combine the information from cytogenetics and molecular changes have been set forth by the European Leukemia Net (ELN) and have culminated in the re-establishment of three risk groups: favorable, intermediate, and adverse categories. Refining prognosis will continue to evolve as the impact of more targets is recognized (Table 20-2).

Treatment

Induction therapy

Treatment for AML generally is divided into remission induction and post-remission therapy. Standard remission induction regimens in the United States for all AML subtypes, excluding APL (see “Acute promyelocytic leukemia” later in this chapter), almost always include 7 days of infusional cytarabine and 3 days of an anthracycline, commonly known as the “7+3” or “3&7” strategy. This strategy results in complete remission (CR) in 70% to 80% of adults younger than 60 years and 30% to 50% of adults with good performance status older than 60 years. The Cancer and Leukemia Group B (CALGB) established that 3 days of daunorubicin and 7 days of cytarabine were more effective than 2 and 5 days, respectively, and that 10 days of cytarabine was not better than 7 days. Also, 100 mg/m² of cytarabine for 7 days was as effective as 200 mg/m² for the same duration. Daunorubicin at a dose of 30 mg/m² was inferior to 45 mg/m², and, more recently, daunorubicin 90 mg/m² has been shown, in large cooperative group trials, to be superior to 45 mg/m² even in selected patients older than 60 years. In a UK study, daunorubicin 60 mg/m² was equivalent to 90 mg/m², establishing 60 mg/m² as an

KEY POINTS

- The most important prognostic indicators in AML are patient age, cytogenetics, and molecular genetics. At diagnosis, check for mutations in *NPM1*, *FLT3* (ITD and TKD), *CEBPA*, *ASXL1*, *RUNX1*, *p53*, *IDH1*, and *IDH2*.
- Complex cytogenetic abnormalities and monosomal karyotypes are associated with poor clinical outcomes.
- t(15;17), t(8;21), and inv(16) are cytogenetic abnormalities associated with favorable outcomes.
- Patients with cytogenetically normal AML and *FLT3*-ITD mutations have an unfavorable prognosis, whereas those with wild-type *FLT3* and mutations of *NPM1* or *CEBPA* have a more favorable prognosis.

Table 20-2 ELN risk stratification by genetics

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); <i>RUNX1-RUNX1T1</i>
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>
	Mutated <i>NPM1</i> without <i>FLT3-ITD</i> or with <i>FLT3-ITD</i> ^{low†}
	Biallelic mutated <i>CEBPA</i>
Intermediate	Mutated <i>NPM1</i> and <i>FLT3-ITD</i> ^{high‡}
	Wild-type <i>NPM1</i> without <i>FLT3-ITD</i> or with <i>FLT3-ITD</i> ^{low†} (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); <i>MLLT3-KMT2A</i> [§]
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); <i>DEK-NUP214</i>
	t(v;11q23.3); <i>KMT2A</i> rearranged
	t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i>
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); <i>GATA2,MECOM(EVI1)</i>
	-5 or del(5q); -7; -17/abn(17p)
	Complex karyotype,§ monosomal karyotype
	Wild-type <i>NPM1</i> and <i>FLT3-ITD</i> ^{high‡}
	Mutated <i>RUNX1</i> ¶
	Mutated <i>ASXL1</i> ¶
	Mutated <i>TP53</i> [#]

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Frequencies, response rates, and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.

*Prognostic impact of a marker is treatment-dependent and may change with new therapies.

†Low indicates low allelic ratio (<0.5); high indicates high allelic ratio (≥0.5); semiquantitative assessment of *FLT3-ITD* allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve “*FLT3-ITD*” divided by area under the curve “*FLT3-wild type*”; recent studies indicate that AML with *NPM1* mutation and *FLT3-ITD* low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic HCT.

‡The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.

§Three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.

||Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosomal abnormality (excluding core-binding factor AML).

¶These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

#*TP53* mutations are significantly associated with AML with complex and monosomal karyotype.

appropriate standard dose and 90 mg/m² as a reasonable alternative in patients with adequate cardiac status.

Current guidelines suggest that patients younger than age 60, who have significant residual disease without a hypocellular marrow on a day 14 (nadir) bone marrow biopsy, should receive reinduction chemotherapy, either repeating “7+3” or using intensive, high-dose Ara-C (HiDAC)-based reinduction.

Remission induction in defined patient subgroups

While “7+3” induction chemotherapy remains the standard treatment for a large subgroup of patients, recent drug approvals have demonstrated improved survival with add-

on and alternative agents in defined patient populations. In patients between the ages of 60 and 75 with therapy related AML, secondary AML, and AML with myelodysplasia-related changes, CPX-351 a liposomal formulation of cytarabine/daunorubicin in a fixed 5:1 molar ratio leads to an increased rate of complete remission and overall survival compared to standard 7+3 induction. For patients between the ages of 18 and 60 with a *FLT3* mutation (ITD or TKD) the addition of the multi-kinase inhibitor midostaurin on days 8 to 21 of induction therapy with 7+3 and consolidation decreased the risk of death by 22% and increased overall survival at 5 years by 7%. The CD33 antibody-drug conjugate gemtuzumab ozogamicin (GO) was recently re-approved by the FDA in combination with 7+3 when given in fractioned doses on days 1, 4, and 7 based

on a randomized study from the French ALFA group. This regimen led to improved event-free survival compared with 7+3 alone in patients with favorable- and intermediate-risk AML. In addition, an individual, patient-level meta-analysis of multiple clinical trials demonstrated improved overall survival in patients treated with gemtuzumab. A recent study suggests that patients with a particular single-nucleotide polymorphism (SNP) in the gene encoding CD33 (CC genotype) have a substantial response to GO. Although this finding needs to be confirmed in additional studies, this SNP may serve as a potential biomarker for the selection of patients with a likelihood of significant response to GO.

Consolidation

Once remission has been achieved, further therapy is required to prevent relapse. Options include repeated courses of consolidation chemotherapy or allogeneic hematopoietic stem cell transplantation (HSCT). The choice of whether to pursue consolidation chemotherapy or HSCT is dependent on balancing the risks of AML relapse with the risks of transplant-related mortality. In general, consolidation chemotherapy is recommended for patients with favorable-risk disease, whereas HSCT at first remission is recommended for patients with unfavorable-risk disease. For patients with intermediate-risk disease, the decision to pursue consolidation chemotherapy or allo-HSCT is individualized. Allogeneic HSCT allows the combination of myeloablative or nonmyeloablative chemotherapy with a graft-versus-leukemia effect from the donor cells. Several studies have prospectively evaluated the role of intensive consolidation with HiDAC. CALGB-randomized patients in first remission are treated with four courses of cytarabine using either a continuous infusion of 100 mg/m² or 400 mg/m² for 5 days or a 3-hour infusion of 3 g/m² twice daily on days 1, 3, and 5. Significant CNS toxicity was observed in patients older than 60 years randomized to the high-dose arm; therefore, this regimen is not recommended for older patients. In patients younger than 60 years, there was a significant improvement in disease-free survival associated with the high-dose regimen. Improvement was most pronounced in patients with favorable cytogenetics, including t(8;21) and inv(16).

Although it has become standard to offer 3 or 4 cycles of HiDAC at 1 to 3 g/m² to younger patients with AML who are not undergoing an allogeneic bone marrow transplant, there are no clear data defining the optimal number or intensity of HiDAC cycles. Randomized trials from the United Kingdom Medical Research Council failed to demonstrate that 3 cycles of HiDAC consolidation were better than 2 cycles. Recent retrospective data

suggest that administering HiDAC on days 1, 2, and 3 leads to a shortened duration of neutropenia.

Randomized studies have not demonstrated that consolidation chemotherapy, in general, is of benefit for patients older than 60 years, but older patients able to tolerate additional treatment often are offered modified dosing of bolus cytarabine or additional courses of "7+3." Maintenance chemotherapy outside of APL has not been adopted. Two pediatric randomized trials from the Leucémies Aiguës Myéloblastiques de l'Enfant (LAME) and the Children's Cancer Group (CCG) failed to demonstrate that maintenance chemotherapy improves outcomes. However, randomized trials using hypomethylating agents or targeted FLT3 inhibitors as maintenance therapy are ongoing.

Several studies of post-remission therapy in AML have compared intensive chemotherapy consolidation to HSCT by assigning younger patients with a human leukocyte antigen (HLA)-matched sibling donor to allogeneic HSCT and randomizing other patients to chemotherapy or autologous HSCT. Meta-analyses have shown that autologous HSCT decreases relapse risk but increases treatment-related mortality compared with chemotherapy consolidation, thus resulting in similar overall survival rates of approximately 40% to 45% at 3 to 5 years. There is no specific indication for using autologous HSCT in any prognostic subgroup, but it continues to be employed in some settings, especially in Europe.

Allogeneic HSCT is probably the most effective antileukemic therapy currently available and offers a combination of the therapeutic efficacy of the conditioning regimen and the graft-versus-leukemia effect from the donor cells. Allogeneic HSCT is, however, associated with significant morbidity and mortality. A comprehensive meta-analysis by Koreth et al of prospective clinical trials of allogeneic HSCT in AML patients in first CR evaluated 24 trials and more than 6,000 patients. In this analysis, allogeneic HSCT resulted in significantly improved 5-year overall survival from 45% to 52% for patients with intermediate-risk cytogenetics and from 20% to 31% in patients with poor-risk cytogenetics. There was no benefit of allogeneic HSCT for patients with good-risk cytogenetics. Retrospective analyses of uniformly treated patients have shown that allogeneic HSCT was also beneficial for cytogenetically normal AML patients with *FLT3*-ITD⁺, *FLT3*-ITD⁻/*NPM1*⁻, and *FLT3*-ITD⁻/*CEBPA*⁻. Other efforts are focusing on the use of alternative donor sources of stem cells to allow allogeneic transplant options for patients without fully matched sibling or unrelated donors. Trials utilizing partially matched related donors, including haploidentical donors, as well as cord blood as sources of stem cells, are under way by national

cooperative transplant groups. Finally, using nonmyeloablative or reduced-intensity conditioning regimens is another way to broaden the application of allogeneic SCT toward patients who may not be medically fit to undergo a full preparative regimen.

KEY POINTS

- Treatment of AML generally involves remission induction followed by post-remission therapy.
- CPX-351, a liposomal formulation of ara-c and daunorubicin shows an overall survival benefit for patients with therapy-related AML and AML with myelodysplasia-related changes.
- Patients with a FLT3-ITD or TKD at diagnosis should have the multikinase inhibitor midostaurin given on days 8 to 21 of induction and consolidation. The role of midostaurin maintenance therapy has not been established.
- The standard of care for induction for all other AML subtypes in adults, excluding APL (FAB-M3), remains 3 days of an anthracycline combined with 7 days of cytarabine.
- Consolidation chemotherapy with 3 to 4 cycles of HiDAC is of particular benefit for patients younger than 60 years with favorable cytogenetics involving CBF [$t(8;21)$] and inv(16)]; The optimal number of cycles of post-remission HiDAC in patients older than 60 years remains to be defined.
- Allogeneic stem cell transplantation appears to be of benefit for AML patients in first remission who have intermediate- or poor-risk cytogenetics.

Measurable residual disease

As the number of tools for detecting residual leukemia has increased, measurable residual disease (MRD; previously referred to as minimal residual disease) has emerged as an independent prognostic factor in AML. Many studies demonstrate that MRD negativity has important prognostic impact. Because of this, MRD assessment is now incorporated into ELN guidelines by including a new response category of “Complete remission with negative measurable residual disease.”

MRD is assessed via multiparameter flow cytometry or real-time quantitative polymerase chain reaction (qPCR), which can identify the persistence of leukemia to a level of $1:10^4$ or $1:10^6$. This compares to a detection-level of $\sim 1:20$ using morphology. Additional newer techniques, such as next-generation sequencing and digital PCR, are under development. Despite the recognition of the importance of MRD assessment, testing has not been standardized. This lack of standardization has affected the generalizability and applicability of MRD testing outside of clinical trials.

In theory, the majority of AML patients should be able to be followed for MRD via multiparameter flow cytometry (MFC). Two different approaches have been developed for assessing MFC MRD: (1) the leukemia-associated immune phenotype (LAIP) approach, which defines LAIPs at diagnosis and tracks these in subsequent samples; and (2) the different-from-normal (DfN) approach, which is based on the identification of abnormal flow profiles at follow-up. The benefit of the DfN approach is that it can be applied if diagnostic information is not available. In addition, DfN allows the detection of new abnormal clones (Because LAIPs are DfN abnormalities the majority of the time, with the use of a large panel of antibodies (at least 8 colors), any differences between these 2 approaches are likely to disappear. The leukemia community awaits standardization of flow MRD monitoring so these tools can be utilized at individual centers.

In patients with a leukemia-associated mutation, MRD can be monitored by PCR at the time of complete remission. The tools used to identify MRD via PCR should have a detection sensitivity of 0.1% or better. Multiple studies have shown that the persistence of *NPM1* mutations and fusion genes *PML-RARA*, *RUNX1-RUNX1T1*, and *CBFB-MYH11* following treatment is a predictor of relapse. Thus, patients with these abnormalities should have a molecular assessment of residual disease. It is important to note that not every mutation identified at diagnosis can be used to follow for MRD. Some mutations, such as *DNMT3A*, *ASXL1*, and *TET2* genes, may represent preleukemic founder mutations and may persist upon achievement of complete remission. The detection of these mutations may not represent the presence of AML MRD and thus may not be of prognostic significance for relapse. Mutations in these genes are known to occur with increasing frequency as some individuals age. Furthermore, several mutations, including *ANKRD26*, *CEBPA*, *DDX41*, *GATA2*, and *RUNX1*, may be mutated in the germ line (and are associated with AML development). These mutations will not correlate with disease burden and the variant allele frequency is expected to remain at $\sim 50\%$ throughout the treatment course; genes mutated in the germ line are not useful for following for MRD. *WT1* expression has been assessed as a marker for MRD. However, the ELN working group does not recommend its use due to low sensitivity and specificity unless no other MRD markers (including flow cytometry) are available for a specific patient. If *WT1* expression is used, the validated *WT1* MRD assay developed by ELN researchers from the peripheral blood should be employed.

MRD analysis is being used to risk-stratify patients in current clinical trials. The optimal test, appropriate

detection sensitivity, and standardization of monitoring across centers are all still required before these tools can be widely used. Furthermore, prospective clinical trials are required to assess whether additional post-remission treatment with chemotherapy, allogeneic transplantation, or other agents will improve outcomes for patients with persistent MRD.

AML relapse

The majority of adult patients with AML experience relapse despite initially attaining CR. The prognosis for patients with relapsed disease is poor, and they should be considered for investigational trials. Most AML relapses occur within 2 years of diagnosis. The duration of first remission is of critical prognostic importance, and patients with an initial CR of <6 months are unlikely to respond to standard chemotherapeutic agents. Patients whose initial CR duration was >12 months may have as high as a 50% chance of responding to a HiDAC-containing regimen, even if they had previous exposure to this agent. Examples of reinduction regimens include cytarabine, etoposide, and mitoxantrone (MEC), fludarabine, high-dose cytarabine and granulocyte colony-stimulating factor priming (FLAG), clorfarabine and cytarabine, and cladribine, cytarabine, and growth factor (CLAG). No combination has proven more effective in the few randomized trials attempted. Patients who achieve a second remission should be considered for standard or reduced-intensity allogeneic transplantation, if possible, because the duration of second remission with chemotherapy alone is generally shorter than with CR1. The prognosis for patients who relapse after allogeneic transplantation is dismal.

Patients with molecularly defined subsets of AML, including those with mutations in isocitrate dehydrogenase 1 (IDH1) or IDH2, may benefit from targeted molecular therapies at the time of relapse. In a nonrandomized study of patients with relapsed/refractory AML with IDH2 mutations, the overall response rate with the IDH2 inhibitor and differentiation agent enasidenib was 40.3%. A true CR was achieved by 19.3% of patients, and the median duration of response was 5.6 months. Based on these data, enasidenib was approved by the FDA for relapsed/refractory IDH2-mutant AML. Similarly, treatment with the recently approved IDH1 inhibitor ivosidenib led to an overall response rate of 41.6%, with 21.6% of patients achieving a CR. The median response duration was 6.5 months. Targeted inhibition of FLT3 with potent FLT3 inhibitors in the relapsed and refractory setting may lead to their preferential use in relapsed and refractory FLT3-mutant AML.

Older patients with AML

Most patients with AML are older than 60 years, and their prognosis is dismal, with median survival times of only 8 to 12 months among the most “fit” patients. Older patients have a high frequency of poor prognostic features, including antecedent hematologic disorders, unfavorable cytogenetics, and multidrug resistance (*MDR1*) phenotypes. Also, older patients are often less able to tolerate intensive chemotherapy because of medical comorbidities, polypharmacy, poor performance status, and limited social support. There is no universally accepted standard of care for the treatment of older patients, but they generally are offered either conventional “7+3” induction, hypomethylators, repeated cycles of low-dose subcutaneous cytarabine, supportive care with antibiotics and transfusions, hospice care, or an investigational trial. Although remission can be attained in ~50% of selected older patients with a good performance status using 7+3, these responses are offset their short duration and by mortality rates of 5% to 20%, with <10% of elderly patients achieving long-term survival.

The use of hypomethylating drugs (azacitidine and decitabine) has become a common treatment strategy for elderly patients or patients who are deemed unfit for traditional cytotoxic chemotherapy based on the findings that these agents can result in bone-marrow stabilization, reduction in transfusion needs, and even complete remission in 10% to 20% of patients. Decitabine is now approved for this indication in Europe and is often used off-label in the United States; azacitidine is approved in the US for patients with AML who have 20% to 30% bone-marrow blasts. There is controversy about how to define unfit patients; previous studies used physician judgment, and more recent studies have employed specific criteria for patients unlikely to benefit from intensive induction.

Major cooperative group or multicenter trials, which generally have focused on patients younger than 75 years old with de novo AML and those having a good performance status, show 3- to 5-year overall survival rates of only 10% to 20%; however, many of these patients are not offered any treatment for AML despite randomized data clearly demonstrating a survival benefit favoring treatment with chemotherapy over supportive care in this population. Clinical experience suggests that quality of life is better for those who achieve CR, but data are sparse. Although there are clearly frail and debilitated older patients who cannot tolerate any treatment, age alone should not be used as the major determinant of treatment because several intensive options, including intensified doses of daunorubicin and reduced-intensity stem cell transplantations, are both feasible and effective in selected patients older than

60 years. Many, if not most, older patients with AML fail to benefit from therapy due to its lack of therapeutic efficacy, not due to intolerable toxicities. Many clinical trials seek to combine novel agents with hypomethylating agents or low-dose cytarabine, such as the addition of targeted therapies with FLT3 and IDH inhibitors. In a phase I/II trial, addition of the B-cell lymphoma 2 (BCL-2) inhibitor venetoclax to azacitidine led to a robust CR/CRi (CR with incomplete hematologic recovery) rate of 61 percent. A randomized, placebo-controlled study of azacitidine vs azacitidine/venetoclax is under way and the results are eagerly anticipated. In addition, umbrella “master” trials, that assign patients to a treatment group based on genetic abnormalities, are underway to accelerate the development of targeted therapies. Older AML patients should be encouraged to participate in clinical trials whenever possible.

Acute promyelocytic leukemia

Acute promyelocytic leukemia (APL) is a subtype of AML characterized by a balanced reciprocal translocation resulting in the fusion of the *PML* gene on chromosome 15 with the *RARA* gene on chromosome 17. Patients with APL commonly present with a distinct coagulopathic syndrome caused by thrombocytopenia, hyperfibrinolysis, and disseminated intravascular coagulation (DIC). APL previously was among the deadliest subtypes of AML but is now the most curable form of AML. The incorporation of anthracyclines in the 1970s, all-*trans*-retinoic acid (ATRA) in the 1980s, and the frontline use of ATRA in combination with chemotherapy, have pushed the CR rate in excess of 90% with cures in 80% of patients with APL. Today, outstanding results are attained using ATRA and arsenic trioxide (ATO) and no chemotherapy in low-risk patients or minimal chemotherapy in high-risk patients.

While APL has seen incredible success over the last decades, there are still issues remaining in this disease that must be addressed. The initial diagnosis of APL must be managed as a medical emergency because early mortality from hemorrhage, differentiation syndrome, and infection still occur at a significant rate. Early suspicion and recognition of APL, prompt ATRA administration, and appropriate transfusion support are critical steps in mitigating early death in APL.

APL exists in hypergranular (typical) and microgranular forms. In hypergranular APL, the promyelocytes are strongly myeloperoxidase-positive and have bi-lobed or kidney-shaped nuclei. The cytoplasm has densely packed, large granules, and characteristic cells containing bundles of Auer rods (faggot cells, named after a British term for the bundle of sticks that these Auer rods resemble) may be

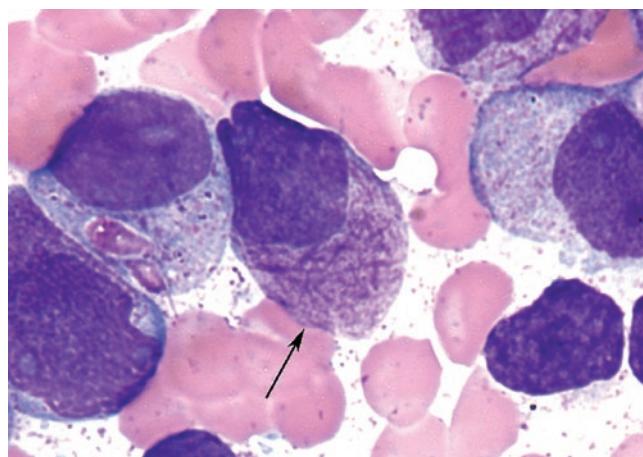


Figure 20-2 A faggot cell. Source: ASH Image Bank/Peter Maslak.

found in most cases (Figure 20-2). Cases of microgranular APL have predominantly bi-lobed nuclei, are strongly myeloperoxidase positive, and often have a very high leukocyte count and doubling time. APL is generally characterized by low expression or absence of HLA-DR, CD34, CD117, and CD11b.

If APL is suspected after initial assessment, ATRA should be initiated immediately at 45 mg/m^2 per day in 2 divided doses. Initiation of ATRA should not wait for confirmation of the disease but instead should be started at first suspicion of APL. Cytogenetics, FISH for t(15;17) or PCR for PML/RARA should be ordered, and results should be expedited to confirm the diagnosis.

In addition to administration of ATRA, it is critical to pay very close attention to hemostatic support. Correction of coagulopathy after ATRA initiation may take several days. We recommend blood work every 6 hours and aggressive transfusion support so that platelets are maintained at $50 \times 10^9/\text{L}$ or higher, fibrinogen at 150 mg/dL or higher, and prothrombin time (PT) and partial thromboplastin time (PTT) are maintained near normal levels. It is also recommended that any invasive procedures, including the insertion of central access catheters, be delayed until coagulopathy has been resolved.

APL promyelocytes have the unique ability to undergo differentiation with exposure to ATRA. Some infrequent APL variants, such as t(11;17)(q23;q21) with ZBTB16-RAR α and cases with STAT5B-RAR α fusions, are resistant to ATRA. Differentiation syndrome (DS) is a complication during induction caused by the effects of differentiating agents (ATRA and ATO) on leukemic blasts. Hyperleukocytosis frequently accompanies DS and may precede the clinical manifestations of DS. DS leads to a systemic

inflammatory response-syndrome-like process. The most common problem seen with DS is acute respiratory distress caused by diffuse interstitial pulmonary infiltrates, which appear as a pleural effusion and pulmonary infiltration on chest imaging. Other features that may occur with DS are weight gain, edema, fevers, acute renal failure, pericardial effusions, and, hyperbilirubinemia. Severe DS can be fatal, and patients with a WBC count greater than $5 \times 10^9/L$ at diagnosis are at increased risk for early mortality.

The use of prophylactic steroids in an ATO- and ATRA-based induction approach is recommended as a mechanism to decrease the risk of severe DS. Additional agents to prevent DS are utilized in patients presenting with leukocytosis, or patients who develop leukocytosis. In the APL0406 trial (a study of patients with low-risk APL; less than 10,000 peripheral white cell count at presentation), patients, whose WBC count exceeded $10 \times 10^9/L$ after ATRA/ATO initiation, received hydroxyurea to reduce the peripheral WBC count. This approach appears to be effective because no deaths from DS occurred on the ATRA/ATO arm. It is therefore recommended that hydroxyurea be started if the WBC count rises over $10 \times 10^9/L$. Patients who present with an elevated WBC count are at higher risk for DS. The APML4 protocol, which included high-risk patients, used up-front idarubicin, in part, to prevent hyperleukocytosis and DS. In this trial no patients, including those with high risk disease, died from DS. Thus, based on WBC count, adjusting induction therapy with an anthracycline is recommended for patients with high-risk disease. On the other hand, hyperleukocytosis that occurs during the treatment of standard-risk APL should be managed with hydroxyurea, reserving anthracycline use for resistant cases. Should DS occur despite these measures, rapid administration of dexamethasone (10 mg twice daily) at the earliest manifestation of DS with continuation until symptoms resolve, and for at least 3 days, can be lifesaving.

Treatment approaches for APL

While combination regimens with ATRA and an anthracycline (with or without cytarabine) induce remission in >90% of patients, and long-term cures are achieved in >70% to 80% of patients in many series, ATRA/ATO-based regimens have virtually replaced ATRA/anthracycline-based induction for patients with low risk APL. In addition to offering a survival benefit when given as consolidation for newly diagnosed patients (as opposed to cytotoxic chemotherapy-based consolidations), arsenic, combined with ATRA, produces high rates of durable CR in newly diagnosed patients with low-risk disease with low rates of hematologic toxicity as compared to ATRA plus an anthracycline. The ATRA/arsenic combination led to a 100%

complete remission rate, a 97% event-free survival, and a 99% overall survival at 2 years. ATRA/Arsenic is now the standard of care for patients with APL presenting with a WBC count of <10,000. Finally, early use of arsenic has also been recognized as contributing to the elimination of maintenance ATRA and chemotherapy in most lower-risk APL patients.

Anthracyclines or gemtuzumab ozogamicin have been used because the APL cells are exquisitely sensitive to these agents. These agents lower the WBC count and therefore directly treat the APL, minimize the risk of DS, and are used in the up-front treatment for high-risk APL. There are multiple approaches to the treatment of high-risk APL. One approach for such patients is combination therapy with ATRA + anthracycline chemotherapy. There is some controversy regarding the best chemotherapy to include with ATRA during induction, but an anthracycline alone appears to be sufficient, and either daunorubicin (60 mg/m^2 for 3 days) or idarubicin (12 mg/m^2 on days 2, 4, 6, and 8) can be used. Consolidation protocols differ between the United States and European cooperative groups but generally include several cycles of anthracycline-based chemotherapy. Patients presenting with high-risk disease, who are treated with ATRA/anthracycline-based induction, may benefit from intermediate-dose cytarabine or HiDAC during either induction or consolidation. However, recently, most patients with high risk APL are offered ATO-based regimens, and the use of intermediate or high dose cytarabine is thus not indicated. Preferred regimens for high-risk disease include ATRA/ATO and either idarubicin or GO for induction therapy. Some protocols for high-risk patients have also incorporated prophylactic intrathecal chemotherapy, though it is not known if this therapy is needed in the era of ATO-based approaches. The role of maintenance therapy is also debated in APL. With these choices and the very good outcomes reported, we strongly recommend that, in order to achieve the expected results, the patient should be treated with one regimen consistently throughout the treatment course and that components not be mixed, for example: induction from one regimen and consolidation from another.

The persistence or reappearance of promyelocytic leukemia/retinoic acid receptor alpha (PML-RARA) fusion-gene transcripts in patients with APL is highly predictive of clinical relapse, and frequent monitoring by RT-PCR has been integrated into most clinical trials. The ideal monitoring approach is not clear because most patients achieving a molecular remission will not relapse and currently monitoring is recommended only for high-risk patients or those who are not able to complete adequate therapy. The chance of recurrence with modern approaches to APL is very rare.

Depending on the time to relapse, ATO with or without ATRA can be considered (because APL may regain sensitivity) as can GO and ATRA/idarubicin. There is currently not a standard approach to relapsed APL with the widespread use of ATO in newly diagnosed patients and the rarity of recurrence. However, we generally recommend another attempt at ATRA/ATO if it has been at least 6 months since the last exposure. In addition, autologous stem cell transplantation can be considered for patients in second complete molecular remission. Allogeneic stem cell transplantation is reserved for patients who are not able to attain a complete molecular remission (CMR) or who are in second relapse.

KEY POINTS

- APL is a unique subtype of AML that is exquisitely sensitive to ATRA, anthracyclines, arsenic trioxide, and gemtuzumab ozogamicin.
- ATRA should be started immediately if the diagnosis of APL is suspected.
- APL may be complicated by a life-threatening coagulopathy or differentiation syndrome.
- In patients treated with ATRA/ATO induction, prophylactic steroids should be used to prevent differentiation syndrome. Should differentiation syndrome occur, patients should be promptly treated with dexamethasone (10 mg twice daily) for at least 3 days.
- Cure rates are high in APL.

Pediatric AML, including Down syndrome

CLINICAL CASE

A 6-year-old boy presents with a 4-week history of fatigue and fever and a 1-week history of bruising and pallor. Laboratory evaluation shows pancytopenia. Bone marrow aspiration shows myeloblasts with granules and an occasional Auer rod. Cytogenetic studies reveal t(8;21).

Pediatric AML has unique clinical features, risk stratification schemas, and therapeutic approaches. Cutaneous involvement is more common in children, particularly in infants diagnosed at younger than 1 year of age. Poor-prognosis cytogenetics are less frequent in children, and, within the pediatric spectrum, age is not a critical prog-

nostic indicator, except for children with Down syndrome. Children may tolerate intensive chemotherapy better than adults, and this tolerance may affect the optimal therapeutic approach. Standard induction chemotherapy in pediatrics typically includes cytarabine and an anthracycline with the addition of a third agent, such as etoposide. Most current pediatric AML protocols use at least four cycles of chemotherapy with HiDAC-based consolidation. Autologous HSCT has been abandoned by most pediatric groups, whereas the role of allogeneic HSCT is highly variable. In North America, most children with favorable features are treated with chemotherapy alone, whereas most children with poor-risk features are offered allogeneic HSCT from either a related or unrelated donor. Children with favorable cytogenetics have an overall survival rate of ~70%, whereas children with adverse cytogenetics have much poorer outcomes. Recently, gemtuzumab was demonstrated to improve event-free survival (EFS) of children with intermediate-risk and high-risk AML. Response to GO correlated with expression of CD33 measured by flow cytometry. Future trials of frontline therapy will continue to assess the benefit of GO, as well as the incorporation of epigenetic agents, such as azacitidine, and targeted therapeutics for specific subtypes, such as FLT3-ITD AML.

Children with Down syndrome have a 46- to 83-fold increased risk of AML and are generally younger than other pediatric AML patients. Down syndrome patients with AML typically acquire FAB-M7 (acute megakaryoblastic leukemia [AMKL]), which is characterized by acquired *GATA1* mutations. AMKL in Down syndrome may be preceded by transient myeloproliferative disorder (TMD), a condition unique to these children. TMD is a clonal disorder characterized by circulating blasts and dysplastic features and usually is diagnosed in the first few weeks after birth. Although TMD typically resolves spontaneously within the first 3 months, intensive supportive care may be required, and early death has been reported in as many as 15% to 20% of cases. For those who survive, ~20% to 30% will later develop AMKL. Children with Down syndrome and AML who are younger than 4 years have better prognosis compared with both non-Down syndrome AML and Down syndrome AML patients older than 4 years at diagnosis. Children with Down syndrome have greater toxicities with treatment and usually are not offered HSCT in first remission.

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21

Acute lymphoblastic leukemia and lymphoblastic lymphoma

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common leukemia in children (representing 23% of all pediatric cancer diagnoses and 76% of leukemias among children <15 years of age) but accounts for only 20% of adult acute leukemias. Lymphoblastic lymphoma is rarer, representing 2% of adult and 30% of pediatric nonHodgkin lymphomas. These entities are closely related biologically and clinically and may share presenting features, although symptoms of bone marrow failure are much more common in ALL. Otherwise, typical cases of lymphoblastic lymphoma with bone marrow involvement exceeding 25% are classified as ALL.

The prognosis for both adult and especially childhood ALL has improved substantially since the beginning of multi-agent curative therapy in the 1970s with the use of risk-directed combination induction-consolidation-continuation (maintenance) regimens that include central nervous system (CNS) prophylaxis. In children, treatment now results in complete remission (CR) rates of 97% to 99%, 5-year event-free survival rates of 80% to 87%, and 5-year survival rates of 90% to 94%. The use of similar treatment regimens in adults with ALL has improved the prognosis, with CR rates of 65% to 95% and 5-year survival rates of 25% to 74%, with more favorable results in younger than in older adults. Several factors contribute to the less favorable prognosis for adults with ALL, including a higher rate of the more therapy-resistant T-cell immunophenotype, a lower rate of favorable genotypes and more frequent high-risk genetics as well as a worse response to initial therapy measured as measurable residual disease (MRD). In addition, older adults also suffer from comorbidities associated with older age that impair the ability to tolerate the intensive multiagent chemotherapeutic regimens that have been used successfully in children. Several studies have shown that the most important factor for the differences in outcome is the different treatment regimens used by pediatric vs adult hematologists and medical oncologists. Differences in treatment adherence may also play a role but may be less pronounced when pediatric oncologists and adult hematologists work closely together.

The treatment and prognosis of lymphoblastic lymphoma mirror those of its leukemic counterpart, but the distribution of immunophenotypes and genetic aberrations, as well as clinical prognostic markers, differs.

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Classification and diagnosis of acute lymphoblastic leukemia

The World Health Organization (WHO) classification, revised several times (fourth edition, 2017), has replaced the older French-American-British (FAB) classification based on morphology and reflects the increased understanding of the biology and molecular pathogenesis of the diseases. The WHO classification divides these heterogeneous lymphoid diseases into two major categories: precursor lymphoid neoplasms and mature lymphoid neoplasms. The precursor lymphoid diseases include both B-lymphoblastic leukemia/lymphoma and T-lymphoblastic leukemia/lymphoma. The WHO classification further subdivides the precursor B-cell acute lymphoblastic leukemia (ALL) cases by recurring molecular-cytogenetic abnormalities to provide prognostic and therapeutic information as well as to facilitate the implementation of specific molecularly targeted therapies (Table 21-1). Burkitt lymphoma is the one subset of ALL that is classified as a mature B-lymphoid neoplasm.

Examination of a bone marrow aspirate is important in the diagnostic evaluation of suspected ALL because as many as 10% of patients with ALL lack circulating blasts at the time of diagnosis and because bone marrow cells tend to be better than blood cells for genetic studies. Fibrosis or tightly packed marrow can occasionally lead to difficulties with marrow aspiration and can necessitate a biopsy to make the diagnosis. In patients with marrow necrosis (<2% of cases), patchy disease or aplastic presentation, multiple and repeated marrow aspirations are sometimes needed to obtain diagnostic tissue.

Immunophenotyping

Flow cytometry at diagnosis

Because the morphologic and cytochemical features of leukemic lymphoblasts are not specific enough for all important diagnostic distinctions, immunophenotyping by flow cytometry is essential for diagnosis. A panel of antibodies is needed to establish the diagnosis and to distinguish among the different immunologic subclasses of leukemic cells. Although ALL can be classified according to the normal sequential stages of normal T-cell and B-cell development, most groups find it therapeutically useful only to distinguish between T-cell ALL, B-cell precursor (BCP) lymphoblastic ALL, and mature B-cell ALL.

Cytoplasmic CD3 is lineage-specific for T-cells, which are also positive for terminal deoxynucleotidyl transferase (TdT) and frequently for the less specific marker CD7. B-lineage cells are distinguished by a combination of at least

Table 21-1 WHO classification of precursor lymphoid neoplasms (B- and T-lymphoblastic leukemia/lymphoma)

B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); <i>BCR-ABL1</i>
B-lymphoblastic leukemia/lymphoma with t(v;11q23); <i>KMT2A</i> * rearranged
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); <i>ETV6-RUNX1</i>
B lymphoblastic leukemia/lymphoma with hyperdiploidy†
B-lymphoblastic leukemia/lymphoma with hypodiploidy‡
B-lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); <i>IL3-JGH</i>
B-lymphoblastic leukemia/lymphoma with t(1;19) (q23;p13.3); <i>E2A-PBX1</i> (<i>TCF3-PBX1</i>)
B-lymphoblastic leukemia/lymphoma with <i>iAMP21</i>
B-lymphoblastic leukemia/lymphoma <i>BCR-ABL1</i> -like§
T lymphoblastic leukemia/lymphoma
NK-lymphoblastic leukemia/lymphoma

*Formerly known as *MLL*.

†>50 chromosomes and usually <66 chromosomes.

‡≤46 chromosomes, often subdivided into: near haploid (23–29 chromosomes), low hypodiploid (33–29), high hypodiploid (40–43), near diploid (44–45). The last category sometimes not counted as hypodiploid.

§“Provisional entity.”

||Rare entity, difficult to distinguish from blastic plasmacytoid dendritic neoplasms, some early T-cell or even AML-entities with few distinguishing markers. Not discussed further.

two of CD19, cCD79a or cCD22. Mature B-cells are surface immunoglobulin-positive and most are also CD20-positive. In addition, many precursor ALLs are CD10-positive B-lineage cells positive for HLA-DR and TdT and both B- and T-lineage cells frequently express CD38. A summary of CD markers and specific immunophenotypic techniques and findings in ALL is found in Chapter 12.

Epidemiology

The distribution of the immunophenotypic subsets differs slightly between adult and pediatric ALL. T-cell ALL accounts for fewer than 10% of children below age 10 and increases with age during adolescence and constitutes approximately 25% of adult ALL, though its incidence decreases again with increasing age. Mature B-cell/Burkitt ALL accounts for ~2% to 5% of adult and pediatric ALL cases, and BCP ALL accounts for the remaining cases. There are also racial or ethnic differences in the distribution, with T-cell ALL accounting for 10% to 12% of white and 25% of black children with ALL.

Immunophenotypes in clinical and genetic subgroups

Infants with ALL, typically with genetic rearrangement of the *KMT2A* gene, usually lack CD10 expression, and the genetic aberration is associated with worse prognosis.

Myeloid-associated antigens may be expressed on otherwise typical lymphoblasts and is associated with common genetic variants such as *KMT2A* rearrangements, *ETV6-RUNX1* fusion, Philadelphia chromosome-positive (Ph+) ALL, and the recently described group with *ZNF384* rearrangements. The presence of myeloid-associated antigens lacks prognostic significance but can be useful in immunologic monitoring of patients for minimal residual leukemia.

Early T-cell precursor (ETP)-ALL has a unique immunologic marker (typically CD1a⁻, CD8⁻, CD52 [dim], and positive for one or more stem cell or myeloid antigens) and gene-expression profile reminiscent of a double-negative thymocyte that retains the ability to differentiate into T-cell and myeloid, but not B-cell, lineages. Clinical characteristics include more frequent chromosomal abnormalities, a higher BM blast count, and a higher risk of CNS involvement at diagnosis compared with non-ETP-ALL/LBL. These cases were initially associated with a dismal treatment outcome with chemotherapy, but recent reports suggest that the adverse outcome may be limited to a higher incidence of induction failure, whereas post-induction outcome may be similar to non-ETP cases with intensive chemotherapy stratified according to MRD. In contrast, ETP-ALL in adult patients appears to have a less favorable prognosis due to lower CR rates and inferior overall survival (OS) compared to patients with non-ETP-ALL/LBL, but small numbers hamper detailed interpretation.

Mature B-cell ALL, Burkitt ALL, has a unique immunophenotype with expression of surface immunoglobulin, strong expression of CD20, negative for TdT, and also has distinctive morphologic and cytogenetic features. These ALLs are associated with chromosome 8 translocations involving the *MYC* proto-oncogene.

Genetic aberrations in the leukemic cells and their prognostic importance

It is commonly agreed that ALL arises from a lymphoid progenitor cell that has sustained multiple specific genetic injuries that lead to malignant transformation and proliferation. Initially these genetic changes were discovered as recurrent cytogenetic aberrations, but, as molecular techniques have been developed, a multitude of submicroscopic changes have been discovered, and a range of different

complementary methodologies are now used for characterization of the leukemic clone.

Compilations of results from multiple studies have, over the years, defined a set of common, non-overlapping genetic alteration groups that are now regarded as separate subtypes of ALL. Some of these alterations have been found to be early initiating events because they were tracked back to neonatal blood spots and were concordant in twins who were both diagnosed with ALL. Other recurrent changes have been shown to be promiscuous between the canonical groups and appear in subclones inconsistently represented at diagnosis and relapse and are therefore considered as contributing to the malignant phenotype, but secondary.

When array techniques became available to characterize expression-patterns, the canonical subgroups of genetic changes were found to match distinctive expression-patterns in unsupervised clustering analyses signifying the universal deregulation of gene expression according to the initiating events despite variations in secondary alterations. Some of these subtypes are now reflected in the revised WHO classification. More than 75% of adult and childhood cases can readily be classified into prognostically or therapeutically relevant subgroups based on the modal chromosome number (or DNA content estimated by flow cytometry), structural mutations, or expression patterns.

Table 21-2 lists selected genetic abnormalities, most of which can be identified by conventional cytogenetic analysis and/or fluorescent in situ hybridization (FISH) with prognostic and therapeutic relevance.

Changes in B-lineage ALL

Ploidy

Hyperdiploidy (also known as high hyperdiploid), defined as involving 51 to 67 chromosomes, is seen in approximately 25% to 30% of childhood cases and in 6% to 7% of adult cases and is associated with a favorable prognosis in childhood ALL and in some studies of adult ALL. High hyperdiploid karyotype may be associated with an increased cellular accumulation of methotrexate and its polyglutamates, an increased sensitivity to antimetabolites, and a marked propensity of these cells to undergo apoptosis.

By contrast, hypodiploidy with <44 chromosomes, especially near haploidy (24–31 chromosomes) and low hypodiploidy (32–39 chromosomes), is consistently associated with an adverse prognosis in both children and adults with ALL. Hypodiploidy at this level is uncommon in both children and adults, accounting for <2% of cases. Among children with hypodiploid ALL, near-haploid ALL cases frequently have alterations targeting receptor tyrosine kinase signaling and Ras signaling (71%), and low-hypodiploid

Table 21-2 Clinical and biologic characteristics of selected genetic subtypes of ALL

Genetic abnormality	Frequency (%)		Estimated event-free survival (%)		Therapeutics
	Adult	Pediatric	Adult	Pediatric	
B-cell					
Hyperdiploidy (>50 chromosomes)	6–7	23–29	30–50 at 5 years	80–90 at 5 years	Antimetabolites
Hypodiploidy (<44 chromosomes)	2	1	10–20 at 3 years	30–40 at 3 years	
t(12;21)(p13;q22)/ETV6-RUNX1 fusion	0–3	20–25	Unknown	85–95 at 5 years	Intensive asparaginase
t(1;19)(q23;p13.3)/TCF3-PBX1 fusion	2–3	4–5	40–70 at 3 years	85–90 at 5 years	High-dose methotrexate
t(9;22)(q34;q11)/BCR-ABL1 fusion	25–30	2–3	40–60 at 2 years	70 at 5 years (DFS)	ABL1 tyrosine kinase inhibitors (imatinib/dasatinib)
t(4;11)(q21;q23)/KMT2A -AF4 fusion	3–7	2	10–20 at 3 years	30–40 at 5 years	Several principles tested
BCR-ABL1-like/Ph-like	Unknown	15–20	Unknown	40–50 at 5 years	Tyrosine kinase/JAK2 inhibitors in some cases
iAMP21	Unknown	2	Unknown	60–70 at 5 years	HR therapy
DUX4-rearrangements (+/– associated ERG-deletions)	5–10	4–5	Unknown	ERG-del “favorable”	Unknown
ETV-RUNX1-like	Unknown	1–3	Unknown	“Few relapses”	Unknown
ZNF384 rearrangements	4–11	1–6	Unknown	Unclear/mixed	Unknown
MEF2D rearrangements	5	1–4	Unknown	72	Unknown
T-cell					
NOTCH1 mutations	60–70	50	~50 at 4 years	90 at 5 years	γ-Secretase inhibitors
HOX11 overexpression	30	7	70–80 at 3 years	90 at 5 years	
HOX11L2	13	20	~20 at 2 years	~45 at 5 years	
t(9;9)(q34;q34)/NUP214-ABL1 fusion	5	4	Unknown	Unknown	ABL kinase inhibitors (imatinib/dasatinib)
t(8;14); t(2;8); t(8;22); c-MYC overexpression	5	2	50–80 at 3 years	75–85 at 3 years	Short-term intensive multiagent chemotherapy with rituximab

cases are characterized by alterations in *TP53* (91%) that are commonly present also in normal cells and that may be inherited.

Flow cytometric determination of cellular DNA content is a useful adjunct to cytogenetic analysis because it is automated, rapid, and inexpensive, and its measurements are not affected by the mitotic index of the cell population; results can be obtained in almost all cases. Flow cytometric studies can sometimes identify a small drug-resistant subpopulation of near-haploid or low-hypodiploid cells that may have been missed by standard cytogenetic analysis. Array techniques (comparative genomic hybridization or single-nucleotide polymorphism arrays) are increasingly used to diagnose hyper- and hypodiploidy. Because of the resulting uniparental disomy or the pattern of chromosomal gain, arrays may also detect near haploid clones that

have duplicated and that are masquerading as diploid or hyperdiploid cases.

Translocations resulting in gene deregulation or gene fusions

Specific reciprocal translocations have important biologic and clinical significance. Some translocations can mobilize strong promoter-enhancer elements like the immunoglobulin heavy- or light-chain gene or the T-cell antigen receptor genes to sites adjacent to a variety of genes resulting in deregulated overexpression. Such translocations occurs in 2% to 3% of B-precursor ALL; the most frequently affected over-expressed gene is *CLRF2*. Another classic example of this type of translocation occurs in Burkitt ALL, in which the transcription factor *MYC* is translocated to the promoter-enhancer element of the im-

munoglobulin heavy- or light-chain and, consequently, is expressed aberrantly.

The genetic rearrangements may also result in the fusion of two genes to form a new oncoprotein, which sometimes has dysregulated transcription factor properties. These chimeric transcription factors may regulate genes involved in the differentiation, self-renewal, proliferation, and drug resistance of hematopoietic stem cells. Included in this group of translocations are those involving the *KMT2A* gene (formerly *MLL*) on chromosome 11q23, the most common of which is t(4;11), which results in the creation of the *KMT2A-AF4* fusion gene. Other fusion genes result in the aberrant activation of tyrosine kinases, which play a critical role in pathogenesis of these diseases.

An important example of this type of translocation is the Philadelphia chromosome, where the t(9;22) results in the *BCR-ABL1* fusion gene and causes constitutive activation of the ABL1 tyrosine kinase, which is directly linked to disease pathogenesis and a worse prognosis. The t(9;22) is highly age-dependent, with children representing 2% to 3% of patients, but with an increasing incidence with age so that about 25% of adults and 50% of patients more than 60 years old are Ph+. The details of treatment and outcome are discussed below.

Other specific fusion-forming translocations involve the *TCF3*-locus on chromosome 19. Approximately 3% of children and 6% of adults harbor the t(1;19), resulting in a *TCF3-PBX1* fusion; very rarely the t(17;19) produces the *TCF3-HLF* fusion gene. The *TCF3-PBX1* was previously associated with poor prognosis, but recent studies have shown excellent results with modestly intensive therapy. However, the prognosis after relapse is very poor. The *TCF3-HLF* cases, on the other hand, have a universally dismal prognosis.

An important translocation resulting in a gene fusion that is almost always submicroscopic is the *ETV6-RUNX1* fusion. This alteration occurs in approximately 20% of childhood cases but is exceedingly rare in adulthood and is associated with good clinical characteristics and outcome.

Intrachromosomal amplification of chromosome 21 (iAMP21)

The iAMP21 subgroup of BCP ALL is one of the newly defined WHO subgroups occurring in 2% of older children and very rarely in adults. It is generated via breakage-fusion-bridge cycles and chromothripsis. The result is the amplification of one part of chromosome 21 and loss of other regions. The amplified part always contains the *RUNX1*-gene, which may serve as a marker and a diagnostic tool, which is easily detectable by fluorescence

in situ hybridization (FISH). The aberration is also easily detectable by array analysis. Patients treated with standard-intensity regimens have fared poorly and have a very high risk of relapse, but stratification to intensive therapy has improved the outcome.

Copy number alterations: important secondary changes

Several genes of importance for leukemogenesis, such as *IKZF1*, *CDKN2A*, *RB*, *BTG1*, *PAR1*, frequently have copy-number alterations in ALL. Most of these cases are interpreted as deletion of a tumor suppressor gene. These alterations do not seem to represent primary events in most cases because they occur across the canonical groups, frequently in subclones, and are inconsistently represented at relapse. Early reports suggested a simple association with poor outcome, but more recent data indicate that a poor outcome applies only to patients with either a slow treatment response or when the *IKZF1* mutation is associated with additional recurrent copy-number alterations and not in combination with the favorable changes (*ETV6-RUNX1*/high hyperdiploidy).

Novel B-lineage subgroups

More recently, the application of genome-wide analysis of gene expression and DNA copy number, complemented by high-throughput sequencing technologies (transcriptome sequencing [mRNA-seq], targeted exome capture, and whole-genome sequencing) and epigenetic approaches, has identified some novel genetic alterations, further reducing the number of cases with unknown genetic background. One such group was initially identified by a distinct expression pattern linked to deletion of the ETS-related gene *ERG*. It was subsequently discovered that the *ERG*-deletions were sometimes subclonal like other copy-number alterations, but that the consistent underlying genetic lesion was a rearrangement of the transcription factor *DUX4* occurring in 4% to 5% of childhood cases. At least the *ERG* deletion has been associated with a good prognosis even in cases with codeletion of *IKZF1*.

Another group, detected by expression pattern, clustered with *ETV6-RUNX1*-fusion cases without having the t(12;21) translocation. Initial studies indicate an incidence of 1% to 3% of childhood cases. The incidence in adults is unknown. Few relapses have been reported, but the prognostic information is incomplete.

The transcription factor *ZNF384* has been described to rearrange together with a number of partner genes. These cases have a characteristic low *CD10* expression and co-express the myeloid markers *CD13* and *CD33*. Rearrangements occur in 1% to 6% of children and 5% to

15% of adult B-lineage ALL. The prognostic significance is, so far, unclear.

Finally, *MEF2D* rearrangements affecting a gene named because of its binding to myocyte-regulating genes. As for the rest of these novel groups, the leukemogenic mechanism for this aberration is unclear. *MEF2D* rearrangements, which occur in 1% to 4% of pediatric and 7% of adult ALL cases, have either a unique expression pattern or cluster with the *BCR-ABL1*-like cases when the partner gene is *CSF1R*. *MEF2D* rearrangement is a marker for worse than average prognosis in a compiled heterogeneous population. *MEF2D* rearrangement was found to be associated with worse than average prognosis in a patient population compiled from several adult and pediatric studies.

***BCR-ABL1*-like ALL**

The first group described as a “provisional WHO-entity” on the basis of gene-expression pattern is the *BCR-ABL*-like group, which was originally described because it clustered with the *BCR-ABL1* translocated cases in expression arrays in the absence of the t(9;22) translocation. The *BCR-ABL*-like group consists of a number of genetic aberrations previously making up a substantial part of the “B-other” group of patients not belonging to any of the previously well recognized subgroups; it occurs in as many as 12% to 14 % of children but in as many as 27% of young adults with ALL. Overexpression of the *CRLF2* gene by several mechanisms, together with deregulated JAK/STAT/EPOR signaling and *IKZF1*-deletion, is common. Both *ABL1* and other similar tyrosine kinases (*ABL2*, *PDGFRB* and *CSF1R*) have been described to form fusions with partners other than *BCR* in this group. The resulting activation of kinases may be clinically actionable by repurposing of drugs approved for other indications, such as the tyrosine kinase inhibitors (TKIs) imatinib, dasatinib, ponatinib, and JAK2 inhibitors. Anecdotal evidence indicates that imatinib and dasatinib can induce remissions in patients with Ph-like ALL and ABL-class fusions that have responded poorly to chemotherapy. Dasatinib is used (nonrandomized) in cases of *BCR-ABL*-like ALL positive for ABL-class fusions in ongoing frontline trials in the Children’s Oncology Group, St Jude’s, and Dana Farber Cancer Institute. The same trial at St Jude’s uses the Janus kinase (JAK) inhibitor ruxolitinib for patients with JAK-STAT (signal transducers and activators of transcription) activation.

Changes in T-cell ALLs

Transcription factors

Subgroups of T-ALL are characterized by the presence of specific chromosomal aberrations leading to ectopic expression of a transcription factor, such as *TAL1*, *TLX1*,

TLX3, or others. Such aberrations can be caused by chromosomal translocations involving one of the T-cell receptor genes, chromosomal rearrangements with other regulatory sequences, duplication/amplification of transcription factors, and mutations or small insertions generating novel regulatory sequences acting as transcription enhancers. Genomic sequencing approaches have identified more than 100 genes that can be mutated in T-ALL. Notably, the majority of genetic alterations that have been identified do not independently predict T-ALL outcome, which is most strongly predicted by assessment of MRD, with few exceptions (listed below).

NOTCH

Constitutive activation of NOTCH signaling, which has important roles in hematopoiesis, angiogenesis, cell proliferation, apoptosis, and T-cell development, is the most common abnormality in T-ALL. Mechanisms of NOTCH activated include mutations in *NOTCH1*, *FBXW7* (15%), or rarely chromosomal translocation t(7;9)(q34;q34.3)), which juxtaposes *NOTCH1* and *TCRB*. *NOTCH1* or *FBXW7* mutations have been associated with a favorable prognosis in adult and childhood ALL. NOTCH signaling can also be activated secondary to alterations in other signaling pathways, including PI3K/Akt/mTOR and c-myc. This has prompted clinical studies with NOTCH inhibitors.

Alterations involving kinases

Another group of genetic changes result in increased kinase signaling, with interleukin 7 (IL7) signaling attracting particular attention because of its role in normal T-cell development. The interaction of IL7 with the heterodimeric IL7 receptor induces Janus kinases 1 (JAK1) and JAK3 phosphorylation and subsequent recruitment and activation of the signal transducer and activator of transcription factor 5 (STAT5). Activating mutations in *IL7R*, *JAK1*, *JAK3* and/or *STAT5* are present in 20% to 30% of T-ALL cases, with a higher representation within the *TLX3*-positive, HOXA-positive, and ETP-ALL patient subgroups. Upon phosphorylation, STAT5 dimerizes and translocates to the nucleus where it regulates the transcription of many target genes, including the anti-apoptotic B-cell lymphoma 2 (BCL-2) family-member proteins. In addition to the JAK/STAT pathway, the RAS-MAPK and PI3 kinase pathways are also activated by IL2, IL7 and SCF that act on the developing T-cells. Notably, the *IL7R* signaling cascade can also be hyperactivated in patients that do not carry genetic aberrations in the *IL7R*, *JAK*, or *STAT5* genes, indicating that still other mechanisms exist to activate this pathway.

The PI3K/Akt/mTOR pathway is also frequently activated in T-ALL, most often caused by inactivation of PTEN

due to *PTEN* mutations or deletions or defects in other signaling pathways that alter *PTEN* transcription or translation. In addition, PI3K/AKT/mTOR can be activated directly by mutations in *AKT1*, *PI3KCA*, *PI3KR1*, and *IL7R*, or indirectly from abnormalities in JAK/STAT, NOTCH, or MAPK. *RAS*, *N-RAS*, and *PTEN* mutations seem to be associated with a poor prognosis.

Epigenetic changes

Recent genomic studies have identified recurrent lesions in genes involved in DNA methylation (*DNMT3A*, *DNMT3B*, *TET1*, *IDH1*, *IDH2*), histone methylation (*EZH2*, *SUZ12*, *MLL1*, *MLL2*, *DOT1L*, *SETD2*, *EED*, *JARID2*, *UTX*, *JMJD3*, *NSD2*), and histone acetylation (*CREBBP*, *EP300*, *HDAC7*, *HDAC5*, *NCOA3*) in T-ALL. There is an indication that epigenetic changes may correlate with poor outcome and chemoresistance. None of the other aberrations has been shown to predict outcome consistently and independently from end-of-consolidation MRD.

The importance of host genomics

Susceptibility to ALL

There are a number of genetic syndromes with a clearly increased risk of ALL, the most common of which is Down's syndrome (discussed separately under treatment). Other susceptibility syndromes include defects in DNA repair, such as ataxia telangiectasia, Bloom syndrome, and others. Because these syndromes also affect the impact of therapy on the host, they are important to diagnose. This is also true for Li-Fraumeni syndrome, which has rather recently been associated with hypodiploid ALL as described above.

More detailed and extensive genetic testing, particularly in familial cases and in patients with unknown genetic conditions, has also revealed new germ-line variants in several genes associated with somatic changes in leukemic cells. Recent genome-wide association studies have also identified germ-line single-nucleotide polymorphisms of several genes that are strongly associated with ALL susceptibility.

Host genome and adverse effects of treatment

Host-genome variants that increase the likelihood of side effects to therapy have also been described affecting for instance the incidence of bone osteonecrosis due to corticosteroid therapy and to pancreatitis as a result of asparaginase-therapy. These genetic variants do not yet influence the routine choice of therapy, but therapy is influenced by differences in nucleoside metabolism:

- Several genetic variants affect the metabolism of thiopurines. A few patients (1 in 300) have an inherited homozygous deficiency of thiopurine S-methyltransferase, the

enzyme that catalyzes the S-methylation of mercaptopurine. Mercaptopurine should be reduced markedly (eg, 10-fold reduction) in these patients to avoid potentially fatal hematologic toxicity.

- Similar severe myelotoxicity has been observed in patients of Asian and Hispanic ancestry with a homozygous variant of the nucleoside diphosphate-linked moiety X-type motif 15 (*NUDT15*) gene. Previously, both a better antileukemic effect and an increased risk of second malignancy were described in patients heterozygous for the *TPMT*-gene variant, but more recent reports have negated these initial findings; therapy should largely be titrated as for wild-type patients. Heterozygous effects of the *NUDT15*-polymorphisms have not been extensively studied.

The Clinical Pharmacogenetics Implementation Consortium has developed guidelines for thiopurine therapy (updates at <http://www.pharmgkb.org>) based on the association between clinical effects and phenotype or genotype of the thiopurine methyltransferase. Guidelines have recently also been updated with some *NUDT15* data.

Prognostic factors

Because ALL is universally fatal if untreated, it is only meaningful to discuss prognostic factors when curative therapy is administered. Such therapy has varied in intensity and has had very different cure rates over time, which means that most risk factors are valid only in the context of a particular therapy. Many clinical prognostic factors were identified early on when patients were first cured of ALL with considerably less intensive therapy than is currently used. Some of these factors have lost their independent prognostic significance as more intensive therapies have been introduced. The discovery of genetic subgroups has further refined the stratification systems because many of these subgroups are also associated with prognosis. However, also the genetic subgrouping has seen the same development over time, with generally decreasing impact when risk-adapted therapy has been implemented. Because of the impact of therapy and the large number of prognostic factors, which sometimes co-vary and interact with each other, all current protocols have utilized more or less complicated algorithms for stratification, taking several of these prognostic factors into account. Recently, attempts to expand the potential of the parameters measured have been incorporated into a more general model, which takes into consideration the full scope of some of the continuous variables. The proposed model is discussed in the MRD section.

Table 21-3 Prognostic factors used for risk stratification

Prognostic factors	Favorable	Adverse
Adult		
Age (y)	<35	>60
Leukocyte count ($10^9/L$)	<30 for B cell	>100 for T cell
Immunophenotype	Thymic T-ALL	Early T-cell precursor (in some studies)
Genotype	High hyperdiploid (in some studies)	<i>BCR-ABL1; MLL</i> rearrangement
		Hypodiploidy <44
Minimal residual disease after induction	Low/absent	High
Pediatric		
Age (y)	1 to 9	<1 or >10
Leukocyte count ($10^9/L$)	<50	>50
Immunophenotype	B-lymphoblastic	T-cell
Genotype	Hyperdiploidy >50; <i>ETV6-RUNX1</i>	Hypodiploidy <40; <i>KMT2A</i> rearrangements, <i>iAMP21, IKZF1</i> deletions or mutations
Minimal residual disease after induction	<0.1%</0.01%/negative	>1%
Minimal residual disease after consolidation	Negative (T cell)	Positive (T cell)

The prognostic impact of genotypes is discussed in the section on genetic alterations. Clinical risk factors and the MRD response are discussed below. Table 21-3 lists some prognostic factors that may be used for risk stratification and/or risk-adapted therapies in current clinical protocols.

Clinical prognostic factors

Age

Children with ALL, aged 1 to 9 years, have a better outcome than either infants or adolescents, who, in turn, fare significantly better than adults. For infants, the prognosis is clearly linked to the genotype rather than with age because infants without *KMT2A* rearrangements have only a slightly worse prognosis than older children do. How much the age-dependent difference in outcome between children and adults depends on leukemia biology and how much depends on differences in administered therapy is unclear, but results from uniform treatment protocols point to a combination effect: risk-group stratified analyses show no differences in some groups but a residual difference in other groups. In protocols including adults, the outcome of therapy worsens with increasing age.

Sex

Male sex is associated with a higher risk profile in many study populations, but, with risk-adapted therapy, the differences in outcome found in early studies are mostly abrogated. Some protocols still stratify boys to longer maintenance therapy. Female sex has, in some studies, been

associated with a higher risk of treatment-related mortality.

Race

Many population-level studies show differences in outcome between ethnic groups. Whites tend to have the best outcome, with inferior results for those of Hispanic, black, and, in some studies, Asian ancestry. In protocol-specific settings, some of these differences are explained by higher-risk characteristics; pharmacogenomic variation and socioeconomic factors have been proposed to contribute by affecting access to care.

Leukocyte count

Leukocyte count is a continuous variable, with increasing counts conferring a poorer outcome in B-lineage ALL. In childhood ALL, there is general agreement to use a presenting age between 1 and 9 years and a leukocyte count of $<50 \times 10^9/L$ as minimal criteria for low-risk B-lymphoblastic ALL; age and leukocyte count have less prognostic value in T-cell ALL. In adult ALL, age <35 years and a leukocyte count of $<30 \times 10^9/L$ are considered favorable prognostic indicators, and a leukocyte count of $>100 \times 10^9/L$ is considered a poor prognostic feature for T-cell ALL in some protocols.

Immunophenotype

T-cell ALL has, in large comprehensive protocols, lost most of its prognostic importance as a high-risk factor, but many

protocols still include some upgrading of the treatment intensity of T-cell patients. However, several studies have shown that T-cell patients with a good response to initial therapy can be treated according to standard-risk protocols.

CNS involvement

CNS involvement at diagnosis is present in 1% to 3% of children (as high as 10% in infants) and in about 5% of adults. CNS involvement (increased cell count of leukemic origin in diagnostic CSF) is associated with an increased risk of relapse, particularly relapses involving the CNS. Most protocols stratify patients with CNS involvement to extra-intrathecal therapy and/or CNS irradiation, and some also increase systemic therapy. The prognostic impact of lower grade (leukemic cells, but no increase in cell number) CNS infiltration is less clear, but a recent study indicates that intensified CNS-directed therapy is probably warranted. The introduction of leukemic cells by a so-called “traumatic tap” (≥ 10 red cells/ μL CSF) with leukemic cells detectable is associated with an inferior outcome and, in most contemporary protocols, to intensified CNS-directed therapy.

Secondary acute lymphoblastic leukemia

Secondary ALL (sALL) following treatment for a primary malignancy is rare compared with secondary myeloid diseases. Data on cytogenetic and molecular characteristics of sALL are limited, with 11q23 abnormalities, mainly t(4;11) (q21;q23) as the most frequent genetic findings. Other translocations included t(9;22)(q34;q11) and t(8;14)(q24;q32). An analysis of the SEER database, evaluating patients with sALL after various cancers or lymphoma with a latency period of at least 12 months, identified 4,124 cases of de novo ALL and 79 cases of sALL. At diagnosis, patients with sALL were significantly older than patients with de novo ALL. While multivariate analysis suggested that sALL is an independent predictor of poor outcome, median survival in both groups was conspicuously low, casting doubt on the generalizability of these findings.

Minimal residual disease detection

The value of MRD as the strongest prognostic factor independent of traditional pretherapeutic risk factors has been shown in both children and adults with ALL. It is mainly risk stratification according to MRD that has led to the reduced prognostic impact of clinical and genetic factors.

The response to initial therapy as assessed morphologically remains an important prognostic marker but is not sufficiently sensitive to accurately assess the depth of response. Sensitive methods for quantification of MRD include molecular analysis of clone-specific immunoglobulin/T-cell

receptor [IG/TR] gene rearrangements or molecular markers, for example, fusion gene transcripts and multiparametric flow cytometry. Recognition of the unique strengths and weaknesses of these methods and awareness that their sensitivity and specificity vary across treatment time points and therapeutic settings are crucial for correct interpretation of MRD data. In addition, MRD levels in BCP-ALL (but not in T-ALL) are typically 1 to 3 logs lower in peripheral blood than in bone marrow, implying that marrow assessments remain crucial in BCP-ALL. Because of the variable limits of detection between different assays and differences in clinical implications of different thresholds, the term “measurable residual disease” instead of “minimal residual disease” may be more appropriate.

Flow cytometry MRD

Multiparametric flow cytometry (MFC) for MRD analysis is based either on the discrimination of ALL cells from normal counterparts or, more precisely, on the identification of the leukemia-associated aberrant immunophenotype (LAIP). LAIP can be identified in more than 90% of patients with ALL, and its detection is relatively easy and fast, although the maximum sensitivity of MFC MRD detection is approximately 1 log lower than that of molecular methods. Another limitation of flow cytometry is the requirement for uniform data interpretation.

Molecular MRD

Detection of leukemia-specific rearrangements of immunoglobulin and T-cell receptor (IG/TR) genes by RT-qPCR is possible in more than 95% of patients with ALL. Sensitivity is determined separately for each assay and routinely reaches 10^{-4} to 10^{-5} (1 leukemic cell in 10,000 to 100,000 normal cells). Initial target identification is laborious, time-consuming, and expensive, but it has been optimized and standardized through the efforts of the Euro-MRD Consortium (<http://www.euromrd.org>), which now includes nearly 60 laboratories worldwide.

Target identification may be facilitated by next-gen sequencing (NGS) techniques covering the same genetic regions. This technology is also being developed for MRD-quantification; it can reach the same or possibly even higher sensitivity and may also have some advantages compared with PCR, for instance, for the detection of emerging subclones. However, the methodology needs validation and standardization before it can be applied routinely.

Specific genetic aberrations applicable to MRD detection are present in about 30% to 40% of B-cell precursor ALL (BCP-ALL) and 10% to 20% of T-cell ALL (T-ALL). Both KMT2A rearrangements and Ph+ ALL may routinely be monitored by this technique. The approach is easier

and less expensive than *IG/TR* rearrangement detection, but interpreting RNA-based results is more challenging than interpreting DNA-based results. Moreover, these two methods may deliver discordant results in a subset of patients as shown in Ph+ ALL, possibly reflecting differences in leukemia stem-cell biology and by changes in the transcriptional activity of the leukemic cells.

MRD for clinical stratification

MRD is used both for stratification of patients to more-or-less-intensive therapy, but it is important to note that different study groups use different cut-off values, depending on the MRD technique, timing of MRD analysis, the therapy administered, and the target patient population.

Most groups consider patients with end-induction MRD <0.01% to be excellent responders, and those with end-induction MRD ≥0.01% as poor responders, but it is also clear that patients with MRD of ≥1% have much worse outcomes than those with lower levels of MRD positivity. Patients who fail to achieve clinical remission (>5% leukemic cells in bone marrow at the end of induction) or who have high persistent levels at later time points (frequently measured at the end of the first consolidation block), may become candidates for allohematopoietic stem cell transplantation (allo-HSCT). The German Multicenter Study Group for Adult ALL (GMALL) demonstrated that patients with molecular induction failure, undergoing SCT in first complete remission (CR1), had a significantly better probability of continuous CR than those without SCT (66% vs 11%). MRD measurement is now used to improve risk stratification and to allocate patients to allo-HSCT in most pediatric trials and in some adult clinical trials.

Recently, immunotherapy with a bispecific T-cell engaging antibody blinatumomab (discussed later in more detail) has been approved by the FDA for MRD-positive ALL, and a trial testing a blinatumomab-chemotherapy combination was recently amended to stratify all MRD-positive patients to receive the drug and only randomizing MRD-negative patients.

MRD in the setting of stem cell transplantation

Results of MRD monitoring after HSCT and its acceptance as a guide to therapeutic intervention are more variable. The historic reliance on chimerism analysis after HSCT is being replaced by evidence that the higher sensitivity and better specificity of *IG/TR*-based MRD testing enables earlier and more specific detection of impending relapse. Patients with evidence of MRD after SCT have significantly worse outcomes compared with patients without evidence of MRD due to a high cumulative incidence of relapse irrespective of whether ALL patients are

classified as high-risk or standard-risk by conventional criteria.

Pre-transplantation MRD levels have also been shown to have prognostic relevance in adult and pediatric patients, although informative thresholds and time-points differ between clinical trials. In a trial conducted by the Italian NILG study group, patients with MRD levels ≥10⁻³ at week 16 and/or week 22 had a higher 6-year relapse incidence than did patients with MRD <10⁻³ (64% vs 23%). The French GRAALL-2003 and -2005 trials showed that SCT benefitted patients with MRD levels ≥10⁻³ at week 6 and that SCT eliminated the unfavorable impact of poor MRD response. In contrast, SCT did not improve outcome in MRD good responders.

MRD integrated with genetic subtype

Opportunities to further refine the prognostic and predictive value of MRD were demonstrated in a recent analysis of 3,113 patients who were treated in UKALL2003 with a median follow-up of 7 years. A detailed analyses of early treatment response was performed in groups of patients who were defined by clinical features, sentinel genetic lesions and MRD, evaluated by analysis of *IG/TCR* gene rearrangements, and considered as a continuous, rather than dichotomized, value. The risk of relapse was correlated with MRD kinetics and was directly proportional to the MRD level within each genetic risk group, but the absolute relapse rate that was associated with a specific MRD value differed significantly by genetic subtype.

A related approach was taken by the French Acute Lymphoblastic Leukemia Study Group (FRALLE) in a study to determine whether oncogenetic mutations, combined with MRD, could improve outcome prediction in pediatric T-cell acute lymphoblastic leukemia. By multivariable analysis, an oncogenetic classifier based on *NOTCH1/FBXW7* mutations and *RAS/PTEN* germ line status, MRD, and white blood cell count were the 3 most discriminating variables independently predictive of relapse. Taken together, these findings indicate that integration of genetic subtype-specific MRD values may allow more refined risk-group stratification in future risk algorithms to more accurately identify patients with the lowest and highest risk of relapse.

In summary, MRD has become a standard procedure to assess the initial treatment response, stratify patients to risk groups (and recently to specific addition of therapy) defined by MRD response, and monitor disease burden in the setting of stem-cell transplantation (SCT) for early recognition of impending relapse and as a potential end point in clinical trials. While MRD levels also correlate with treatment outcome at the time of second remission and before allo-HSCT for relapsed leukemia in pediatric and adult ALL, it

is considerably less predictive of long-term leukemia-free survival (LFS) than in the setting of first-line therapy.

Treatment of ALL

Treatment of B-precursor ALL and T-ALL in children

Usually, childhood ALL cases are divided into low- (standard) risk, high- (intermediate or average) risk, and very-high-risk groups, although the US Children's Oncology Group advocates four categories, including a very-low-risk group.

In the United States, the risk groups tend to be sequenced into separate trials after initial work-up. The result of the stratification is an observational trial in itself.

In Europe and elsewhere, as well as in some groups in the US, there is a tradition of constructing a comprehensive treatment protocol, which includes diagnostics, stratification, and therapy for all risk groups. However, infants are often treated with separate regimens as are children with Ph⁺ ALL after the introduction of tyrosine-kinase inhibitors.

While risk-directed therapy is the fundamental principle underlying therapy for childhood ALL, there is no consensus on the risk criteria or the terminology for defining prognostic subgroups. Some of the prognostic factors are present at diagnosis, whereas others are the result of genetic analyses which become known during the first weeks of therapy. In addition, other important stratifying factors, such as early response and MRD, become known only after evaluation of initial therapy. As a consequence, all current protocols include a more or less complex stratification system in which the final risk groups may be identified a few months into the therapy.

Treatment for lower-risk groups typically consists of a remission-induction phase, an intensification (consolidation) phase, and prolonged continuation (maintenance) therapy to eradicate residual disease. A delayed intensification phase is often inserted before maintenance, at least for medium-risk patients. Higher-risk patients are, in some protocols, subjected to intensive block therapy after induction before continued therapy with either continuous more standard elements or allogeneic stem-cell transplant for selected subgroups. CNS-directed therapy is started early and is given for different lengths of time, depending on the patient's risk of relapse and the intensity of the primary systemic treatment.

Remission induction

Rates of CR range from 97% to 99% with contemporary chemotherapy. The induction regimen usually contains three

or four drugs, typically a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine, and either asparaginase or an anthracycline. Four-drug inductions commonly include all these drugs from the beginning, sometimes with the addition of cyclophosphamide for higher-risk patients. The intensive chemotherapy is, in some protocols, preceded by a prephase of a single corticosteroid to reduce the leukemic cell burden. The response to this prephase is also used for stratification. The efficacy of prednisone and dexamethasone is dose-dependent. Although both drugs yielded comparable results when given in equivalent doses, dexamethasone still appears to yield improved CNS control and is used preferentially in post-remission therapy in current clinical trials. However, if dexamethasone is used at higher doses ($10 \text{ mg/m}^2/\text{day}$) in induction, this intensification has, in some studies, offset the reduced relapse-rate by an increase in induction deaths, deaths in remission, and worse outcome after relapse. Of the various anthracyclines given to patients with ALL, none has proved superior to any other; however, daunorubicin is used most commonly.

The pharmacodynamics of asparaginase differ by formulation, and, in terms of leukemic control, the dose intensity and duration of asparaginase treatment (ie, the amount of asparagine depletion) are far more important than the type of asparaginase used. Because of the lower immunogenicity, less frequent dosing, and feasibility in intravenous administration of PEG-asparaginase (a polyethylene glycol form of the *Escherichia coli* asparaginase) compared with the native product, PEG-asparaginase has replaced native *E. coli* asparaginase as the first-line treatment in most protocols, but availability of the pegylated product is a limiting factor in some countries.

Immunoreactivity against asparaginase is a significant problem and may cause allergic reactions as well as silent inactivation of the drug. Most major allergic reactions to both native and pegylated asparaginase seem to be associated with inactivation, but not all antibody formation causes inactivation of asparaginase activity, and allergy-like reactions are sometimes not associated with inactivation. For this reason, all patients with significant suspected allergic reactions should be tested for asparaginase-activity after the offending dose. If no activity is detected, patients should be treated with the alternative product derived from *Erwinia chrysanthemi*. It is a clinical decision whether to continue with the pegylated product after pre-medication with antihistamine and steroids if activity is still adequate. Standard monitoring of asparaginase activity with subsequent possibility of dose adjustment has been shown to be of benefit in some protocols, and further trials are ongoing, both to avoid over-treatment and to detect silent inactivation, which should also indicate a change in product used.

Consolidation and delayed intensification therapy

At the end of induction, most protocols have a point of response evaluation and stratification. In some protocols, risk-adapted therapy diverges, whereas some protocols have a common start of post-induction therapy to allow for MRD evaluation. Although there is no dispute about the importance of this treatment, there is no consensus on the best regimen and duration of treatment. Many protocols continue with a therapy element developed by the Berlin-Frankfurt-Münster consortium (BFM-IB protocol) with cyclophosphamide, 6-mercaptopurine, and repeated 4-day blocks of injections of cytarabine, whereas other regimens include high-dose methotrexate with mercaptopurine or regimens based on a lower dose of methotrexate. Patients with a poor response to therapy are, in some protocols, shifted to more intensive, block-based therapy. Delayed intensification (or reinduction), also first introduced by the BFM, is a widely used approach consisting of a repetition of therapy similar to the first remission induction therapy approximately 3 months after the end of remission induction. Delayed intensification has been repeated (double-delayed intensification) in studies with somewhat conflicting results, probably reflecting the treatment stratification and the context of the therapy. Extended asparaginase therapy, starting during induction or early postinduction therapy, has received increasing attention and is under study in a randomized fashion in several protocols. Early results from one such study fail to repeat the benefit of prolonged continuous asparaginase exposure from previous trials, indicating that this benefit may be context dependent.

Maintenance (continuation) therapy

A combination of methotrexate administered weekly and 6-mercaptopurine (6MP) administered daily constitutes the standard continuation regimen for ALL. In some protocols, boys have been treated with a longer duration of continuation therapy than girls because, in the past, male sex has been associated with a poorer prognosis. With improved outcome, both boys and girls are now treated with the same duration of 2 to 2.5 years of continuation therapy in most, but not all, clinical trials. The administration of methotrexate and mercaptopurine, titrated to preset limits of tolerance (as indicated by a range of leukocyte count depression), has been associated with improved clinical outcome. Many investigators advocate that the drug dosage be adjusted to maintain leukocyte counts $<3 \times 10^9/L$ and neutrophil counts between $0.5 \times 10^9/L$ and $1.5 \times 10^9/L$ to ensure adequate dose-intensity during the continuation treatment in childhood ALL, yet not induce excessive myelosuppression. Overzealous use of mercaptopurine is counterproductive, however, resulting in interruption of

chemotherapy because of neutropenia and reduction of overall dose intensity. Furthermore, longer duration of the maintenance phase has been associated with the development of secondary MDS and AML, which may be the true limiting factor for optimizing the use of this element. Pharmacological monitoring of maintenance has been attempted, but so far it has been difficult to replace clinical titration. However, a recent study has identified 6-thioguanine (6TG), bound to DNA, as a potential pharmacodynamic target to aim for. DNA-6TG has been thought to be responsible for the antileukemic effect of both 6MP and 6TG and, importantly, in this study, increasing level of DNA-6TG correlated with a continuously decreased risk of relapse. An ongoing pilot study aims to affect the DNA-6TG incorporation by adding a small dose 6TG to standard maintenance therapy. Piloting as well as toxicity monitoring is important for these patients because prolonged use of standard doses of 6TG during maintenance has previously been associated with profound thrombocytopenia, portal hypertension, and an unacceptable rate of hepatic veno-occlusive disease.

There is no strong evidence for a difference in outcome if the mercaptopurine is taken in the evening or the morning, but it should be taken daily at a fixed time-point to facilitate compliance and should not, for pharmacokinetic reasons, be taken with milk or milk products. The coadministration of food does not seem to influence the outcome if the therapy is titrated to adequate myelosuppression. Although methotrexate is used orally in most clinical trials, parenteral administration could circumvent problems of decreased bioavailability and poor treatment adherence, especially in adolescents. Antimetabolite treatment should not be withheld because of isolated increases of liver enzymes; such liver toxicity is tolerable and reversible.

Intermittent pulses of vincristine and a glucocorticoid have improved the efficacy of antimetabolite-based continuation regimens and have been adopted widely in the treatment of childhood ALL. In a large intergroup randomized trial featuring intensive reinduction, the addition of six pulses of vincristine and dexamethasone during early continuation treatment failed to improve the outcome for children with intermediate-risk ALL, but it has also been shown to be of benefit in some recent studies. Thus, whether this pulse therapy is necessary in contemporary regimens featuring early intensification of therapy is still unclear.

CNS-directed treatment

CNS irradiation

Prophylactic cranial irradiation was an instrumental part of the early success of combination trials with curative

intent and thus became the standard treatment. However it is now being replaced by intrathecal and systemic chemotherapy to reduce radiation-associated late complications. Many protocols have omitted irradiation for most patients but still prescribe irradiation for higher-risk patients. However, the results from several protocols show that prophylactic cranial irradiation can be omitted safely in all patients in the context of effective intrathecal and systemic chemotherapy; subsequently, several protocols without irradiation are currently recruiting patients.

When a radiation dose of 12 Gy is used, it appears to provide adequate protection against CNS relapse even in high-risk patients (eg, those with T-cell ALL and leukocyte counts $>100 \times 10^9/L$).

A meta-analysis of T-cell ALL showed no conclusive evidence to suggest that treatment strategies including CNS irradiation (either prophylactic for all or only for risk groups or patients with frank CNS involvement) had better outcomes than with therapies completely omitting irradiation. These results emphasize the importance of systemic and intrathecal therapy also for patients at highest baseline risk. Another recent meta-analysis including more than 16,000 patients, treated between 1996 and 2007 comparing comprehensive pediatric treatment protocols with and without CNS irradiation, found an increased risk of relapse in the small group of patients with overt CNS involvement at diagnosis but a high rate of events even in the irradiated group. The analysis concluded, in the overall assessment, that CNS irradiation did not affect the overall risk in contemporary protocols.

Systemic chemotherapy

Systemic treatment, including high-dose methotrexate, intensive asparaginase, dexamethasone, and optimal intrathecal therapy, is important to control CNS leukemia. A recently closed very large ($>1,800$ patients) study of T-cell ALL in children and young adults (1 to 30 years of age) from the Children's Oncology Group has randomized high-dose methotrexate (HDM) (protocol M) vs a cycle of Capizzi-style interim maintenance (increasing intravenous methotrexate without rescue, intrathecal methotrexate, vincristine, and asparaginase). The randomization was performed in the context of a backbone protocol including low-dose cranial irradiation (12 Gy) for almost all patients. In this setting, the Capizzi group fared better with an increased event-free survival (EFS) (88.9% vs 83.3%) compared with the HDM group. The study also randomized the addition of nelarabine to both arms throughout postinduction therapy for medium- and high-risk patients in a factorial design; nelarabine improved the EFS for both groups, partly by reducing the number of CNS-involving relapses.

Intrathecal chemotherapy

Triple intrathecal therapy with methotrexate, cytarabine, and hydrocortisone is more effective than intrathecal methotrexate alone in preventing CNS relapse, but it may not improve the overall outcome. A meta-analysis showed that adding intravenous methotrexate for patients treated with triple intrathecal therapy improves outcome by reducing both CNS and non-CNS relapses. Because the presence of ALL blasts in the cerebrospinal fluid, even from traumatic lumbar puncture, has been associated with an increased risk of CNS relapse and poor EFS, special precaution should be taken to decrease the rate of traumatic lumbar punctures (eg, transfusion to increase platelet count to $\geq 50 \times 10^9/L$ for initial intrathecal treatment, having the most experienced clinician perform the procedure with the patient under deep sedation or general anesthesia), and intrathecal therapy should be intensified in patients with blasts in the CSF even if these are due to a traumatic lumbar puncture. Patients should remain in a prone position for at least 30 minutes after the procedure to enhance the distribution of the chemotherapy within the CSF and to avoid post-spinal headache.

Hematopoietic stem cell transplantation

With the generally improving results in primary treatment, the indications for HSCT in first remission have become more exclusive. In practice, the rate of HSCT in first remission varies among different protocols.

A very poor early response to remission-induction treatment, possibly with the exception of patients aged 1 to 6 years with favorable leukemic cell genetics (mostly high hyperdiploidy), is an indication for transplantation in many, but not all, protocols. There is more consensus regarding patients with remaining detectable MRD at high level after consolidation, who are uniformly considered to have an HSCT-indication. Few convincing results indicate that cytogenetic changes only (without taking response to therapy into consideration) should indicate HSCT in first remission. Except in some small studies, transplantation failed to improve the outcome for infant patients with *KMT2A* rearrangement. Hypodiploid cases did not appear to benefit from transplantation, but the number of patients treated with this modality was very small.

BCR-ABL1-positive ALL was, before the advent of TKI therapy, a certain HSCT indication, but also, in this genetic subgroup, transplantation is reserved for poor responders.

New modalities of immunotherapy may further reduce the fraction of B-lineage patients that will be transplanted, but, for poorly responding T-cell patients, HSCT will probably remain the best option for some time. It has been long debated whether children have to be conditioned

with total body irradiation (TBI). A randomized international study has been launched to address this important issue because TBI remains a major source of serious late effects after transplantation.

Special subgroups of ALL in children

Down syndrome

Patients with Down syndrome (DS) have a 10- to 20-fold higher relative risk for leukemia, and they constitute ~2% of pediatric ALL. These patients have the same age range as does the general pediatric population, with the exception of a lack of cases in the infant age group. ALL patients with DS have a much lower incidence of T-cell and mature B-cell ALL and have a low frequency of other specific genetic subtypes of precursor B-cell ALL, but they have a high frequency of activating somatic *JAK2* mutations, affecting approximately 20% of the cases. A compilation of data from several study groups showed that as many as 69% of DS cases have *CRLF2* rearrangements, some of which co-occurred (about 21% of all cases) with activating *JAK2* mutations. Although the outcome has improved with modern treatment, these patients still fared significantly worse than other children with ALL, likely because of a combination of reduced tolerance to chemotherapy, such as dexamethasone and methotrexate, resulting in reduced compliance to protocol treatment, but also to excessive treatment-related deaths. Another possible contributing factor is the paucity of genetic changes associated with better prognosis in this patient population. The *JAK2/CRLF2* alterations themselves do not seem to confer an adverse prognosis compared with other children with DS. However, in a recent Dutch/UK study, *IKZF1* deletions were found in 35% of all patients, and patients with such deletions had a very high risk of relapse, with an EFS of only 21% to 45% in the different national cohorts studied.

Infant ALL

Infant ALL accounts for 2% to 3% of childhood ALL and is characterized by a high frequency of 11q23 chromosomal abnormalities and rearrangements of the *KMT2A* gene (70% to 80%), a CD10-negative pro-B immunophenotype, a tendency towards hyperleukocytosis, CNS involvement, and an inferior outcome. Large collaborative studies are necessary to study this rare subset of patients, but despite very large consortium efforts, progress has been modest at best over the last 15 years, with overall survival hovering between 50% and 60%. New approaches are desperately needed.

Adolescents and young adults

Increasing age is one of the most important poor prognostic factors of outcome in newly diagnosed patients with

ALL. Age is obviously a continuous variable, but treatment strategies and outcomes are often considered according to specific age brackets. While the outcome of elderly patients with ALL is particularly poor, a substantial drop in survival probability compared with pediatric patients is already apparent in adolescents and young adults (AYA). Reasons for this disparity between children and the AYA group, commonly considered as 16 to <40 years of age, include a higher proportion of unfavorable and, more importantly, a lower proportion of highly favorable genetic subtypes (eg, hyperdiploidy, favorable trisomies of chromosomes 4, 10, and 17, the RUNX-ETV1 translocation), psychosocial issues affecting compliance, and lower enrollment into clinical trials as compared with younger pediatric patients.

Nevertheless, large cooperative trials have demonstrated considerably better survival of AYA patients with more intensive “pediatric-inspired” treatment regimens, although these comparisons, retrospectively analyzed, showed survival rates of 60% to 65%. In contrast, when the same age group was treated in adult cooperative-group ALL treatment trials, survival rates have been only 30% to 40%.

The major differences between the adult and pediatric regimens are the more intensive use of nonmyelosuppressive agents (glucocorticoids, asparaginase, and vincristine) earlier and more intensive CNS-directed therapy and more prolonged maintenance therapy as is typically used in the pediatric regimens. It is controversial whether differences in adherence to protocol therapy among pediatric and adult medical hematologists and their patients also play a role. The upper age limit for patients also differs between studies investigating pediatric-inspired regimens in AYA patients.

Several new prospective European and American comparison studies that apply the pediatric approach to AYA trials recently have demonstrated significantly higher survival in adolescents below 21 years who were enrolled on pediatric vs adult trials. The Spanish PETHEMA group demonstrated 6-year EFS and OS rates of 63% for young adults aged 19 to 30 years, suggesting pediatric therapy was advantageous in ALL patients up to 30 years. A similar French trial for patients with BCP- and T-ALL achieved 42-month EFS and OS rates of 55% and 60%, respectively, in patients aged 15 to 45 years. The UKALL-2003 protocol integrated AYAs up to the age of 24 into a pediatric protocol. The 16- to 24-year-olds had a 5-year EFS of 71% and OS of 72%. The Nordic NOPHO-group integrated young adults (18 to 45 years) into the NOPHO ALL-2008 protocol resulting in a 5-year EFS of 74% and an OS of 78%. Some groups expanded the age limit for adults on such pediatric-based therapy to 55 years. In these studies, increasing age beyond 45 years was associated with greater toxic-

ity, in particular, asparaginase-associated pancreatitis, hepatic toxicity and venous thromboemboli. Intensive glucocorticoid and vincristine dosing was also more poorly tolerated in adults as compared with children. While the upper age limit for patients likely to benefit from pediatric-inspired regimens is not clear, these intensive treatment protocols have resulted in better outcomes for young adults with ALL. Study center experience with comprehensive care teams that specialize in the treatment of these patients is essential for achieving the best possible outcome.

Treatment of B-precursor ALL and T-ALL in fit younger adults

In contrast to pediatric ALL, the majority of adult patients with B- and T-cell ALL has, in the past, been treated with less specific consideration of biologic risk. More recently, MRD has gained importance as the most relevant stratification parameter. Two different treatment strategies are widely employed: the Berlin-Frankfurt-Munster (BFM-type) therapy initially developed by the pediatric Berlin-Frankfurt-Münster Group (BFM Consortium) and the hyperfractionated cyclophosphamide, vincristine, adriamycin, and dexamethasone (Hyper-CVAD) regimen pioneered by MD Anderson Cancer Center. CNS-directed prophylactic therapy is a critical element of all ALL regimens, even though numerous variations have been adopted over time and by different study groups.

Therapy derived from pediatric (BFM Consortium) protocols

Treatment of adults with this type of therapy has in general followed the same basic strategy of multi-agent induction, consolidation-intensification, CNS prophylaxis, and maintenance therapy that has been used so successfully in pediatric ALL. The relative contribution of each of these phases toward improved prognosis and disease curability has not been determined rigorously in adult ALL. Use of these intensive chemotherapy regimens has resulted in complete remission rates of 75% to 90%, although cure rates historically were only in the range of 30% to 40% overall. These lower survival rates in adult ALL prompted investigations into the use of allo-SCT in CR1, and results of these studies are reviewed in this chapter. Current clinical research efforts are focused on better risk stratification with implementation of biologically directed therapies tailored to specific disease subsets.

Induction phase

Over the past 20 years, intensification of the induction regimen for adults with ALL has resulted in significant improvement in CR rates, with >80% of patients achiev-

ing remission in many current multicenter studies. A more recent goal of induction therapy is achieving a good molecular response or molecular CR, which is usually evaluated within 6 to 16 weeks of starting therapy.

Building on a backbone of vincristine, a glucocorticoid (prednisone or dexamethasone), and often asparaginase, the addition of an anthracycline (daunorubicin or doxorubicin) has resulted in improved CR rates ranging from 72% to 92%. Dexamethasone is often preferred to prednisone because it penetrates the blood-brain barrier and also acts on quiescent leukemic blast cells (LBCs). Given the high CR rate observed with these 4-drug induction regimens, it has been difficult to demonstrate further improvements in overall CR rates with the addition of other drugs, such as cyclophosphamide or cytarabine, during induction. The Italian Gruppo Italiano Malattie Ematologiche Maligne dell'Adulso (GIMEMA) reported that, similar to childhood ALL, a good response (decrease in circulating blasts to <1,000/mL) to 1 week of pretreatment prednisone before chemotherapy was predictive of a longer CR duration and survival.

L-asparaginase is the only ALL-specific chemotherapy drug, which acts by depleting the serum asparagine levels; it is now also being increasingly used in adults. Pegylated asparaginase (PEG-Asp) has the advantage of a significantly longer asparagine depletion time. Pioneered in the treatment of pediatric ALL, asparaginase contributes to increased response rates and duration of response in adults; however, the reason is not clear because there are no randomized studies supporting its use in adult patients. The toxicities of asparaginase in adults include pancreatitis, hepatotoxicity, and coagulopathy. A study by the Cancer and Leukemia Group B (CALGB), now known as the Alliance, 9511, with the long-acting asparaginase, pegaspargase, showed that patients who achieved effective asparagine depletion had a superior outcome compared with patients who did not achieve asparagine depletion. Ongoing trials by the German Multicenter ALL (GMALL) group of pegaspargase suggest a potential survival benefit in older adults with ALL when the drug is administered at slightly lower doses than have been used by the pediatricians.

The goal of using granulocyte colony-stimulating factor (G-CSF) is to shorten the period of neutropenia to prevent possibly fatal infections, and previous studies demonstrate the utility of this drug with induction regimens for ALL. In the Leucémie Aigüe Lymphoblastique de l'Adulte (LALA)-94 trial, patients were randomized to receive G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), or no colony-stimulating factor (CSF). When given on day 4 of induction until return of absolute neutrophil count of 1,000/mL, patients receiving G-CSF had

significantly shorter hospital stays, a shorter time to neutrophil recovery, and fewer severe infections as compared with patients who did not receive G-CSF. The CALGB 9111 trial highlighted the benefit of using this drug in patients prone to have difficulty with hematologic recovery, specifically older patients. The study observed a trend toward increased CR rates in patients 60 years or older in the G-CSF arm compared with the placebo arm. Although G-CSF does not affect DFS or overall survival (OS), it appears to be safe and also enables patients to proceed with post-remission therapy.

Consolidation therapy

Traditionally, agents similar to the four or five drugs used during remission induction, with the addition of antimetabolites, such as methotrexate, mercaptopurine, or thioguanine, are used for post-remission treatment. The rationale to use systemic high-dose (HD) therapy is particularly to reach sufficient drug levels in sanctuary sites, such as the CNS. Most protocols employ 6 to 8 courses which contain either HD methotrexate or HD cytarabine \pm asparaginase. HD cytarabine is usually administered for 4 to 12 doses at 1 to 3 g/m² and methotrexate at 1 to 1.5 g/m² and as high as 3 g/m². The post-remission treatment modules in adult series typically have been modeled after the pediatric regimens. Cyclophosphamide, high-dose cytarabine, and etoposide also have been incorporated into many post-remission strategies, although it has been difficult to analyze critically the contribution of each drug or schedule to outcome in adult ALL series.

Although induction chemotherapy leads to CR rates that are >90% in many series, the relapse rate in adult ALL patients with adult intensive regimens has been 50% to 75%, leading to many variations of post-remission consolidation treatment in an attempt to eradicate MRD and improve disease-free survival (DFS). Adult consolidation regimens have evolved from pediatric schedules that have been shown to be successful. Post-remission therapy in ALL can include a wide range of drugs, including cytarabine, etoposide, teniposide, methotrexate, mercaptopurine, and thioguanine. In addition, the use of autologous HSCT (auto-HSCT) and allo-HSCT has been incorporated into ALL treatment, as will be discussed in a separate section.

The CALGB compared a more intensive consolidation regimen that included both early and late intensification using eight drugs with previous CALGB trials in a phase 2 study. The results showed that median remission duration improved to 29 months, whereas median survival extended to 36 months. The Italian GIMEMA group conducted a study that included randomization of 388 patients to post-remission intensification followed by maintenance

chemotherapy vs early maintenance therapy without intensification.

In summary, all of these regimens result in similar DFS rates of ~30% to 40% in adult patients with ALL who are entered into cooperative group trials. Outcomes vary considerably, however. Younger patients with favorable-risk cytogenetics can have DFS rates of ~60%; in contrast, older adults defined as more than 60 years old still have a dismal prognosis, with <10% to 15% achieving long-term survival.

Stem cell transplantation in adults

Indication for HSCT

Traditionally, the translocations t(9;22) and t(4;11) were uniformly acknowledged to define a high-risk population with a clear indication for allogeneic HSCT. In addition, patients with high risk features, as defined somewhat differently by various cooperative study groups, were considered candidates for HSCT, whereas most groups did not consider an allogeneic transplant in CR1 for standard-risk patients. In contrast, the UKALL XII/E2993 study, conducted by the MRC and ECOG, observed a significantly superior survival in standard-risk patients who underwent matched related SCT in CR1, with allocation made on a donor vs no-donor basis, whereas high-risk patients did not have a benefit in terms of OS because of toxicity, despite a lower relapse rate. As subsequent studies using pediatric-inspired intensified regimens demonstrated increasingly good chances of cure with chemotherapy alone in adult patients in CR who also displayed an optimal MRD response. European study groups, other than in the UK, do not consider conventionally defined standard-risk ALL to be an indication for HSCT.

In some recent studies, MRD has replaced traditional risk factors as criteria for transplant vs no-transplant decisions. Several prospective trials have shown that 50% to 70% of adult patients with Ph-negative ALL achieve and maintain a good MRD response with chemotherapy, suggesting that early MRD negativity may override adverse clinical or even genetic risk factors. Conversely, patients who remain MRD positive, including many standard-risk patients, clearly benefit from HSCT. Therefore, MRD good-responders should probably not be exposed to the risk of transplant-related mortality (TRM) from HSCT, whereas patients at higher risk of relapse based on high or persistent MRD need to be considered for HCT or experimental therapies. Outcome after HSCT appears to be better in the absence of MRD, suggesting that additional therapy prior to transplant may improve results of MRD, but this notion has not been formally proven. Caveats for these MRD-based approaches include differences in technical

aspects of MRD assessment, selection criteria for MRD-directed therapy, and differences in protocol design. Thus, not all study groups have replaced the conventionally defined high-risk category with a MRD high-risk category.

In current MRD-based strategies, approximately 20% to 30% of MRD-negative patients relapse. In this event, salvage therapy should be employed as a bridge to HSCT. More ideally, MRD-guided interventions should be employed to treat molecular failure prior to hematologic relapse.

Stem cell transplantation in elderly patients

Despite the substantial transplant-related mortality and morbidity reported for elderly ALL patients, the poor outcome associated with nontransplant approaches justifies considering HSCT on a case by case basis. Reduced intensity conditioning (RIC) regimens is comparable to myeloablative conditioning in terms of OS because, generally, lower TRM compensates for higher relapse rates. TBI-based conditioning with 8Gy is tolerable in elderly patients and will be prospectively evaluated in a randomized trial in the UK. The Acute Leukemia Working Party (ALWP) of the European Group for Blood and Marrow Transplantation (EBMT) analyzed a cohort of 142 elderly patients (median age, 62 years; range, 60 to 76 years) who underwent allogeneic HSCT in CR1 using reduced intensity conditioning (RIC). The cumulative incidences of relapse and nonrelapse mortality (NRM) at 3 years were 40% and 23%, respectively, and 3-year OS was 42%.

Maintenance therapy

The rationale behind the use of maintenance treatment is the elimination of slowly growing subclones that persist after induction and consolidation treatments by exposing them to antimetabolite drugs over long periods of time, ranging from 18 months to 3 years after initial diagnosis. Commonly used components of maintenance therapy include daily mercaptopurine and oral weekly methotrexate, which, in some regimens, is supplemented by monthly pulses of vincristine and corticosteroids. Periodic intrathecal methotrexate is employed universally during maintenance. In one randomized study, the maintenance arm with reinforcement cycles was not superior to conventional maintenance therapy (37% vs 38% at 8 years). A treatment duration of 2.5 to 3 years is optimal and is usually recommended.

Despite the lack of randomized trials investigating the importance of maintenance treatment in adults with ALL, two older trials showed inferior results compared with historical controls when maintenance therapy is not included. Thus, on the basis of these data and the clear success of prolonged maintenance therapy in pediatric studies, main-

tenance regimens mimicking those used in pediatric protocols routinely are incorporated into the treatment regimens of adult B- and T-cell ALL.

Hyper-CVAD

An alternative treatment regimen known as Hyper-CVAD was developed at the MD Anderson Cancer Center and uses hyperfractionated cyclophosphamide, dexamethasone, vincristine, and doxorubicin without asparaginase during induction. The regimen employs an extended consolidation in which the induction treatment is repeated during cycles 3, 5, and 7, alternating with high doses of methotrexate and cytarabine in cycles 2, 4, 6, and 8. This is accompanied by rigorous CNS prophylaxis using intrathecal chemotherapy and followed by prolonged maintenance with 6-mercaptopurine (Purinethol), vincristine (Oncovin), methotrexate, and prednisone (POMP regimen). In the trials, more than 90% of patients achieve CR with 3-year survival rates of 50%. Similar to BFM-style regimens, the addition to Hyper-CVAD of rituximab for CD20 positive patients and nelarabine for T-ALL patients has been associated with improved outcomes in phase 2 studies, but the results of randomized trials with these agents are awaited.

Subset-specific treatment

CD20-positive ALL

The B-lineage differentiation antigen CD20 is expressed on ALL blasts of approximately 40% of patients with B-cell precursor ALL and was associated with an adverse prognosis. Despite the caveat that CD20 is not expressed during the most immature stages of differentiation, this provided the rationale for several studies adding the anti-CD20 monoclonal antibody rituximab to standard frontline chemotherapy for both younger (up to 60 years) and elderly patients with BCP-ALL. Across several single-arm studies using different chemotherapy regimens (Hyper-CVAD or GMALL-based) and in a recent large, confirmatory phase 3 trial (GRAALL-2005), addition of rituximab improved treatment outcomes in younger patients. EFS in the GRAALL study improved by 13% from 52% to 65% at 2 years. Toxicity was mild, but an increased rate of infectious events was noted among elderly patients. Thus, rituximab may now be considered the standard of care for patients less than below 55 to 60 years with CD20-expressing ALL.

T-lineage ALL

Nelarabine is a purine nucleoside analog prodrug of 9-β-D-arabinofuranosylguanine (AraG), which is cytotoxic to T lymphoblasts in micromolar concentrations and has

been the only addition to the therapeutic armamentarium for precursor T-cell ALL in recent years. Nelarabine has demonstrated promising single-agent activity in T-ALL, with a 55% response rate in relapsed/refractory T-ALL. This activity and the dismal outcome of salvage therapy for recurrent T-ALL have provided the rationale for investigating nelarabine to optimize frontline treatment strategies for high-risk patients. Addition of nelarabine to an intensive chemotherapy backbone in pediatric patients with newly diagnosed, high-risk T-ALL was well-tolerated, with a 5-year EFS significantly higher than historic controls. Nelarabine has recently been shown to add benefit for intermediate- and high-risk patients in a large randomized study of children and young adults up to the age of 30. The UKALL14 trial is investigating the value of nelarabine added to frontline therapy for adult T-ALL; the results are eagerly awaited.

Elderly and frail patients

Elderly fit patients

Prognosis and principles of treatment

The outcome of elderly patients with ALL continues to be very poor. In the UKALL XII/ECOG 2993 trial, 5-year survival of patients aged 56 to 65 years was only half that of younger adults (21% vs 41%). In even older patients, survival is dismal. Induction mortality is high despite improvements in supportive care, including rigorous prophylactic antimicrobial prophylaxis and use of hematopoietic growth factors. Reasons for the intolerance of intensive therapy in older patients include comorbidities, differences in pharmacodynamics and pharmacokinetics, and a higher prevalence of unfavorable genetic features. As a result, elderly patients are often not considered for intensive induction therapy or allogeneic stem cell transplantation, and they are less frequently enrolled in clinical trials. There is, therefore, no standard chemotherapy treatment for older patients with ALL, and new approaches are needed. In deciding on the best approach to elderly patients, the treatment goals should be guided by patient preference; in advising the patient, one should consider the biological, rather than the chronologic, age, disease risk, performance status, and comorbidities. Objective geriatric assessments of patient fitness and comorbidity scores have been developed but are not yet widely adopted in routine clinical practice. The overall therapeutic strategy may need to be modified during therapy either towards a less ambitious goal or, conversely, to intensification including HSCT if the patient's condition improves when CR is achieved. The transplant option is discussed in the section on HSCT for adult patients.

Treatment options

A corticosteroid prephase, possibly combined with cyclophosphamide or vincristine, should be performed in all patients during the initial diagnostic work-up. CNS evaluation needs to be done in all patients at the time of diagnosis, and prophylactic intrathecal chemotherapy is essential to prevent CNS relapse, whereas cranial radiation therapy is not recommended. The best regimen for induction and consolidation is unknown. The European Working Group for Adult ALL (EWALL) developed a consensus treatment protocol for older patients with ALL that is based on a dose-reduced pediatric (BFM)-based chemotherapy regimen, while the Hyper-CVAD regimen developed by MDACC is often used in the United States. Both of these regimens achieved CR rates in the 70 to 80% range; long-term outcome decreased with age and, overall, was disappointing. Both of these regimens serve as chemotherapy backbones for the addition of immunotherapeutic agents and novel agents.

Different strategies to reduce toxicity of induction to prevent early mortality while maintaining efficacy have been examined. Liposomal anthracyclines have yielded mixed results, and anthracyclines are probably best eliminated from regimens for older patients. Asparaginase has significant morbidity during induction, but this can be mitigated by delaying its use to post-remission therapy.

Targeting CD20 with the naked antibody rituximab failed to show any benefit in older patients in contrast to younger cohorts. The antibody-drug conjugate (ADC) inotuzumab ozogamicin (IO) was combined with reduced intensity mini-Hyper-CVAD in a study in elderly patients that showed encouraging results, but IO is currently approved only for relapsed ALL patients and is not available for frontline therapy outside of clinical trials.

The bispecific T-cell-engaging antibody blinatumomab has shown considerable efficacy in relapsed or refractory and MRD-positive B-lineage ALL. It is not approved for newly diagnosed ALL but has recently gained approval for patients who are MRD-positive. Several trials in the US (MDACC, NCI) and in Europe are evaluating the use of blinatumomab in combination with chemotherapy in trials directed at, or including, elderly patients. In the absence of a trial, administration of blinatumomab in elderly patients who are in CR but who remain MRD-positive after conventional age-adapted induction therapy may be considered.

Frail patients

For those patients older than 75 years and/or with a poor performance status, no standard therapy has been defined and study data are lacking. Purely palliative therapy

is very unlikely to be of any benefit; an attempt should be made to give some form of low-intensity chemotherapy. In the absence of novel low-intensity regimens, corticosteroids and vincristine are reasonable options as long as there is heightened awareness and avoidance of peripheral neuropathy. Mercaptopurine, possibly combined with weekly low-dose methotrexate, can be given in addition (POMP regimen) if organ function permits. CNS prophylaxis in this elderly population should at least be considered, based on individual fitness.

CNS prophylaxis in adults

Risk and diagnosis of CNS involvement

Although <10% of adults with ALL present with CNS involvement, CNS relapse occurs in 35% to 75% of patients at 1 year if prophylactic CNS-directed therapy is not incorporated into treatment. A lumbar puncture at the time of ALL diagnosis is always performed in pediatric studies but is variably timed in adult ALL regimens. CNS disease is present when more than 5 leukocytes per microliter of cerebrospinal fluid are seen along with the presence of lymphoblasts in the cerebrospinal fluid. Symptoms may include headache, meningismus, fever, or cranial nerve palsies. Some patients, however, have no symptoms. Risk factors for CNS involvement in adults include mature B-cell ALL, high serum lactate dehydrogenase levels (>600 U/L), and the presence of a high proliferative index at diagnosis (>14% of lymphoblasts in the S and G₂/M phase of the cell cycle). If symptomatic CNS disease is present at diagnosis, such as focal cranial nerve palsies, concurrent radiation therapy and intrathecal chemotherapy are used.

Combined-modality prophylaxis

The combination of intrathecal methotrexate and 24-Gy cranial irradiation was tested in an early adult trial which demonstrated that CNS prophylaxis reduced the CNS relapse rate at 24 months from 42% to 19% when compared with no CNS treatment. The long-term effects of combined chemotherapy plus cranial irradiation in adults are less well studied than in children in whom combination treatment has well known long-term toxicities including seizures, early dementia, cognitive dysfunction, and growth retardation. Combined radiation and intrathecal chemotherapy in adults can cause substantial acute toxicities that may delay post-remission consolidation treatment. A study by the German GMALL investigators attempted to circumvent these delays by postponing CNS-directed radiation, but this postponement approach led to higher CNS relapse rates of 9% vs 5%. Overall, the use of cranial irradiation as part of primary prophylaxis is losing favor.

Prophylaxis without irradiation

An alternative strategy that relies on intrathecal chemotherapy without radiation has been investigated. This treatment regimen includes so-called triple therapy that uses intrathecal methotrexate, cytarabine, and corticosteroids without irradiation.

CNS relapse rates as low as 5% have been achieved without irradiation by using combination intrathecal treatment in conjunction with high-dose systemic treatment that can penetrate the cerebrospinal fluid. Although CNS-directed prophylactic therapy is essential in ALL treatment, there is no single modality or combination that has been proven to be superior.

BCR-ABL1-positive ALL

Principles of therapy

The frequency of Ph⁺ ALL increases with age and is found in approximately 50% of patients with B-cell precursor ALL over the age of 60, whereas it is uncommon in pediatric ALL patients. Despite similarities in treating children and adults with this disease, the biology of the leukemia differs between these age cohorts. The clinical relevance of this finding primarily concerns differences in the indication for allogeneic stem cell transplantation. Treatment and outcome of patients with *BCR-ABL1*-positive ALL has changed dramatically during the past decade with the addition of the ABL-directed tyrosine kinase inhibitors to frontline therapy.

As with all other subtypes of ALL, the administration of effective CNS-directed prophylaxis to prevent CNS relapse is of critical importance. Available data suggest that intrathecal therapy can be sufficiently effective, with no need for cranial irradiation.

Tyrosine kinase inhibitors

TKIs are an integral part of frontline treatment, either alone (plus corticosteroids) or added to frontline chemotherapy. They should be started as soon as the diagnosis of Ph⁺ ALL is established, in adult patients, typically within 5 to 7 days of presentation. CR rates exceed 90% in nearly all studies irrespective of which TKI is used; TKI, in combination with chemotherapy, reduces MRD more rapidly and to lower levels than is achieved by chemotherapy alone.

The optimal choice of TKI remains to be resolved. With imatinib-based therapy frontline therapy followed by allo-HSCT, DFS rates of 60% to 75% have been reported. Second generation TKI, with more data available for dasatinib than nilotinib, have the theoretical advantages of greater potency and clinical activity against a broader panel of kinase domain mutations conferring resistance. However, no prospective comparative trials have been performed to

determine whether any TKI is superior, and comparisons with historical imatinib-based studies are inconclusive, particularly in patient groups in whom HSCT is an option. For nontransplanted adult patients, the current consensus position is that TKI should be continued indefinitely, if possible.

The third-generation TKI ponatinib has attracted particular interest not only because of its overall potency against the BCR-ABL kinase but because of its ability to inhibit BCR-ABL harboring the T315I TKD mutation, which confers resistance to all other clinically approved ABL-TKI and is the TKD mutation most frequently associated with resistance to dasatinib. Combined with the Hyper-CVAD regimen for frontline treatment of patients with Ph⁺ ALL, ponatinib induced deep molecular responses in the majority of patients and was associated with excellent outcome even in patients not undergoing allogeneic SCT in the only study published to date. These data are particularly relevant for elderly patients with *BCR-ABL1*-positive ALL in whom allo-HSCT may be perceived to pose too great a risk. Longer follow-up will be needed, however, to confirm these promising results. Randomized comparative trials to compare regimens incorporating ponatinib or other TKIs are in preparation.

Chemotherapy regimens and dose intensity

Because up-front TKIs are so effective in inducing CR, the intensity of induction chemotherapy can be reduced without compromising the response rate, while decreasing toxicity at the same time. In a large randomized trial conducted by the French GRAALL Study Group, more intensive induction was actually detrimental in terms of morbidity and mortality and had no survival benefit. Irrespective of age, an initial cytoreductive 5- to 7-day prephase using corticosteroids (CS) is administered to reduce the leukemic cell burden while awaiting the results of molecular classification. An induction cycle combining TKI with CS or CS plus vincristine has been adopted among others by the GIMEMA and EWALL consensus protocols for elderly Ph⁺ ALL, respectively. With this approach, induction mortality can be nearly abrogated even in a multicenter setting.

In contrast to the largely uncontentious principles underlying induction therapy, it is less certain how best to maintain remission. TKI remain a central pillar of post-remission therapy, while it is somewhat controversial whether to continue consolidation with low-intensity or intensive chemotherapy or allogeneic hematopoietic stem cell transplantation (HSCT). More recently, autologous SCT has been reconsidered as an option for a select subset of patients with a very good response to induction therapy. The impact of ponatinib-based therapy on these treatment decisions remains to be determined. In the only study published to date

that prospectively evaluates ponatinib with chemotherapy (using the Hyper-CVAD regimen), 2-year EFS was 81%. Longer follow-up will be needed to determine whether this combination may be curative in a sizeable proportion of patients not undergoing HSCT. Long-term outcome data relying solely on TKI therapy or TKI plus only CS and/or mild chemotherapy are lacking. A high relapse makes such a nonintensive regimen an unattractive option for patients eligible for intensive treatment.

Indications for HSCT for Ph⁺ ALL in adults

Allogeneic HSCT is the best established curative therapy for Ph⁺ ALL and the gold standard against which other forms of treatment should be compared. The limitations of donor availability have been largely abrogated by bigger registries and haploidentical HSCT. Age, comorbidities, and performance status remain critical determinants in the decision to proceed or not to proceed to HSCT and will have to be judged for each patient individually because the risk of nonrelapse mortality (NRM) associated with transplant remains considerable.

A small proportion of Ph⁺ ALL patients, specifically those with a very good molecular response, may remain in remission for prolonged periods. These results have given rise to the notion that patients with low level or negative MRD may not need to undergo HSCT to be cured, although a large proportion of MRD-negative patients will eventually relapse. This issue is most pressing in patients at higher risk of TRM due to age or comorbidities in whom the superior anti-leukemic efficacy of HSCT may be outweighed by early mortality. The use of MRD to inform a treatment decision for or against HSCT is further compounded by the lack of methodological standardization and of universally agreed MRD thresholds, which are also likely to depend on the clinical setting, including transplant modality. The use of autologous SCT for patients with a good MRD response should presently be limited to clinical trials. Overall, these transplant-related questions need to be resolved in prospective comparative trials with sufficiently long follow-up. Patients treated outside of clinical trials should be assessed frequently for MRD, and rising levels should prompt an intervention, including checking for presence of TKD mutations, reconsideration of HSCT, and/or intervention with a non-TKI modality, eg, blinatumumab. Donor lymphocyte infusions have had very limited, if any, success in preventing relapse, probably due to the typically rapid relapse kinetics of Ph⁺ ALL.

Post-transplant TKIs in adults

Whereas TKIs are used universally as part of therapy leading up to HSCT, the role of TKI administration after

transplantation has been studied less extensively, and the overwhelming body of data is based on use of imatinib. In a large retrospective analysis by the BMT and the majority of small prospective trials, use of imatinib after HSCT was associated with a lower relapse rate and better outcome compared with historic controls. The only randomized clinical trials addressing post-transplant TKIs demonstrated excellent long-term survival with both a prophylactic and a pre-emptive MRD-triggered administration of imatinib. Thus, one of these two approaches should be considered as the standard in the post-transplant setting. MRD should be monitored frequently, preference should be given to BM as a source of material, and close attention should be paid to the assay sensitivity.

In patients not undergoing HSCT, TKIs should be given indefinitely as maintenance therapy, even in case of prolonged undetectable MRD.

Ph⁺ ALL in pediatric patients

In pediatric patients with BCR-ABL1-positive ALL, survival has improved from about 40% to approximately 70% with the use of imatinib. The SCT-rate has varied between studies, and there is general agreement that high MRD should be used to select patients that should be transplanted, but the background data for MRD-based stratification is not perfect and will be continuously monitored in planned studies. The addition of TKIs to intensive high-risk chemotherapy has been associated with considerable treatment-related mortality, and studies are planned to randomize patients between backbones of different intensity to complement the TKI therapy, somewhat akin to adapted therapy for the elderly. So far, post-HSCT TKI therapy has been limited (to about a year) in the pediatric setting.

Treatment of relapsed Ph⁺ ALL

Relapse remains the main cause of treatment failure in patients with Ph⁺ ALL, and, if occurring during TKI therapy, is most often associated with presence of a TKD mutation. Such mutations may predate the start of TKI treatment but are not identified by routine methodologies for mutational analysis. However, rising levels of BCR-ABL transcripts should prompt mutation analysis and an appropriate intervention to prevent modification of therapy before overt hematologic relapse occurs because the latter carries an ominous prognosis with median survival of about 6 months.

Switching to a (different) second- or third-generation TKI (eg, dasatinib, nilotinib, or ponatinib) depends on which TKIs were used previously and the result of mutational analysis. Switching is recommended but is likely to be of only short-term benefit.

Immunotherapy strategies are the same as for other B-lineage ALL and are discussed in that section.

Acute leukemias of ambiguous lineage and mixed-phenotype acute leukemia

Definition and epidemiology

In a rare (<4%) subset of acute leukemias now classified as acute leukemias of ambiguous lineage (ALAL) in the revised fourth edition of the WHO classification, more than one lineage can be assigned to the leukemia. Most subentities under this umbrella are labeled mixed-phenotype acute leukemia (MPAL) with addition of a specification describing their lineage mix, ie, B/myeloid (the largest subgroup), T/myeloid MPAL, and MPAL, not otherwise specified (NOS), but acute undifferentiated leukemia also belong under ALAL. The WHO classification uses a limited set of lineage markers in conjunction with genetic drivers and/or a clinical context clearly defining a leukemia entity; thus, the diagnosis of ALAL generally also requires absence of a genetic driver mutation that defines a recognized WHO leukemia diagnosis. However, some rearrangements that act as leukemogenic drivers, for example, in addition, *BCR-ABL* and *KMT2A* rearrangements are consistent with a diagnosis of ALAL (called MPAL with *BCR-ABL1* and *KMT2A* rearranged, respectively). Overall, MPALs are frequently associated with adverse genetic features.

Therapy for ALAL in adults

Adult patients with ALAL have a worse prognosis than other AML or ALL cohorts, and the clinician is faced with the dilemma of whether to choose an AML- or ALL-type regimen as frontline treatment. Most studies addressing this issue demonstrated better results with ALL-like induction or combined ALL-AML therapies. Tyrosine kinase inhibitors should be added for BCR-ABL-driven leukemias.

Allogeneic SCT in CR1 is recommended as the default option in patients with ALAL, based on a more favorable outcome compared with chemotherapy alone that was not limited to very young patients. In patients who have relapsed and reacheived remission, alloSCT in CR2 may yield similar results to their non-ALAL counterparts with AML or ALL.

ALAL in children

In pediatric ALAL, a recent report compiling the experience from 575 cases treated in 24 countries revealed a few important conclusions: As in adults, patients responding to ALL therapy fared better than patients treated with AML-style treatment, and this difference was particularly pronounced if the patients were at least partly positive for CD19. HSCT did not confer an obvious advantage to patients responding to ALL therapy, whereas patients treated with AML-style therapy appeared to have an advantage with transplantation.

Treatment for relapse

Epidemiology and risk factors at relapse

Relapse occurs in 20% to 60% of adult patients and less than 10% of pediatric patients after current frontline treatment protocols, depending on protocol and age group.

Treatment results are much worse at relapse than at primary diagnosis, and it seems as if differences in prognoses evident at primary diagnosis between age groups are even more pronounced at relapse.

There is also a difference in organization. Several large pediatric-relapse programs exist; the ongoing IntReALL study, for instance, includes participation from 19 countries on three continents with common relapse protocols; protocols for relapsed ALL are also organized by the Children's Oncology Group, whereas there is no commonly accepted standard salvage therapy for adults, perhaps reflecting the much worse outcomes in older age groups.

Risk factors for treatment failure are quite different in the relapse setting. In both children and adults, time-to-relapse is the strongest predictor of failure. Particularly, relapse within 18 months from diagnosis, and while the patient is still on intensive chemotherapy, is associated with a dismal prognosis. It is also generally agreed that T-cell immunophenotype is associated with increased risk; protocol strategies in pediatrics have also identified site of relapse as important for outcome. An isolated bone-marrow relapse is worse than combined bone-marrow and extramedullary relapse, which, in turn, is worse than relapse in an isolated extramedullary site (CNS, testes, lymph nodes, liver, spleen, skin, or other organs).

MRD is useful also in the relapse setting not only to assess response to therapy. Although extramedullary relapse can occur without obvious marrow disease, many occurrences are associated with MRD in the marrow. CNS relapses are associated with a higher level of MRD in the bone marrow than in testicular relapses. Importantly, submicroscopic bone marrow involvement at a level of 0.01% (10^{-4}) or higher by PCR at the time of overt extramedullary relapse confers a worse outcome than in cases where bone-marrow MRD is negative.

Relapse treatment in children

Chemotherapy and HSCT indications

In children, cure may be achieved with intensive chemotherapy alone, particularly in B-lineage, late, combined, and extramedullary relapses. Remaining MRD after initial therapy is used to select patients for HSCT in B-lineage cases of late bone-marrow and combined relapses and MRD-negative cases go on to intensive chemotherapy, followed by continuation maintenance as in the primary protocols.

All high-risk cases (all very early isolated BM-relapses and all BM-involving relapses in T-cell ALL) have HSCT as a first option and a poor outcome if transplantation cannot be achieved. HSCT is also prescribed for early extramedullary and combined relapses.

Many protocols have so far advocated the addition of prophylactic cranial irradiation for patients treated with chemotherapy, for patients not receiving total body irradiation in their conditioning for HSCT, and also for patients without CNS involvement because of the higher risk of CNS recurrence in this situation and the overall higher risk of therapy failure.

Management of extramedullary disease

Isolated late extramedullary relapse with MRD-negative bone marrow at relapse diagnosis has the best outcome, also without HSCT. Relapses in sanctuary sites (the CNS and testicles) may be seen as a failure of standard therapy to adequately reach these sanctuaries and which may be rescued with local therapy with irradiation, although systemic therapy has to be administered as well.

The outcome of isolated CNS relapse depends partly on duration of CR1 and partly on whether CNS irradiation was previously performed. Outcome is worse if irradiation has already been used in primary therapy.

For patients with bilateral testicular relapse, local irradiation (22 to 26 Gy) is usually recommended. In patients with unilateral testicular relapse, some leukemia therapists advocate unilateral orchietomy with reduced irradiation (15 to 18 Gy) to the "uninvolved" testicle, but others would rely on intensive chemotherapy alone to spare testicular function. In a recently published compilation of cases from the Children's Oncology Group, similar overall survival rates were reported between patients treated with and without the use of any testicular irradiation.

Treatment of relapse in adults

Systemic chemotherapy

Allogeneic HSCT is the only realistically curative option in relapsed adult patients, but cure is realized in only a minority of patients. If more than 18 months has elapsed since achieving CR, a repeat of the same initial induction regimen is warranted to achieve a second remission. Other commonly used options include high-dose cytarabine combined with an anthracycline or mitoxantrone, the FLAG-Ida regimen (fludarabine, high-dose cytarabine and filgrastim with idarubicin), or HD-MTX plus HD-AraC-based treatment blocks are commonly used salvage regimens for R/R ALL. Clofarabine, a novel purine nucleoside analog, is approved for relapsed ALL in children, but its use in adults as a single agent or in combination is

less well studied. Another agent with some activity in relapsed ALL is liposomal vincristine (Marqibo), which has been approved for adult patients with a second relapse of their disease. In patients with relapsed or refractory T-ALL, nelarabine, a deoxyguanosine analog prodrug, is approved as single-agent therapy with proven favorable results. The CALGB used nelarabine to treat relapsed and refractory patients and demonstrated a CR rate of 41% and OS rate of 28% at 1 year. These results are especially impressive given that many of the patients had failed two or more inductions or had not achieved CR with their last induction regimen. A German study with nelarabine showed similar results. Despite this difficult patient population, nelarabine allowed patients to proceed to transplantation and achieve increased survival. Overall, chemotherapy as first salvage therapy induces a second CR in only about 40% of cases, with a median CR2 duration of about 3 months, median OS of about 6 months, and a 3-year survival rate of 11%.

For patients without histocompatible related donors, transplantation of stem cells from cord blood or marrow from matched unrelated donors has yielded encouraging results. Outcome may be further improved by a new strategy using a reduced-intensity conditioning regimen and selection of donor-derived alloreactive natural killer cells or selective depletion of α/β T cells from the graft used in haploidentical transplantation.

CNS and extramedullary relapse

CNS relapse in adults is associated with a very poor prognosis and often precedes systemic relapse. Median survival is in the range of months. Cranial nerves are often affected so rapid action is essential to preserve neurological function. Intrathecal chemotherapy with dexamethasone, methotrexate, and cytosine arabinoside, in addition to cranial radiation therapy (24 to 30 Gy), is the initial mainstay of treatment. Intrathecal administration does not necessarily achieve sufficient drug concentrations at the base of the brain; concentrations may be improved by intraventricular administration via an Ommaya reservoir. Additional systemic treatment employs high-dose methotrexate and cytarabine as CNS-penetrating drugs in patients with Ph⁺ ALL. Dasatinib has shown some activity and can be added to the above interventions. All patients should be considered for allogeneic HSCT if possible.

Extramedullary relapse, other than in the CNS, usually involves soft tissue, lymph nodes, and skin, may occur concomitantly with or herald systemic relapse, and is a relevant clinical problem particularly after HSCT. Systemic chemotherapy, followed by allogeneic HSCT, including second HSCT if possible, is the most effective therapeutic strategy. Although blinatumomab has shown lower efficacy with ex-

tramedullary (EM) sites, a treatment attempt is warranted. In patients with Ph+ ALL, TKIs are added after appropriate selection, based on prior therapy and mutational status.

It is noteworthy that isolated CNS and EM relapse may occur despite MRD negativity in BM analysis or complete donor chimerism after HSCT.

Immunotherapy

Immune-directed chemotherapy

IO, a CD22 antibody conjugated to calicheamicin, an enediyne antitumor antibiotic, has shown a composite CR rate of 49% in a single-institution study for rel/ref B-ALL whether given on a weekly or bimonthly schedule. IO has shown activity in adults with relapsed/refractory ALL, including those enrolled in a global, open-label, phase 3, randomized trial (INO-VATE). In this trial, 326 adult patients with relapsed or refractory ALL were assigned to receive either IO or standard intensive chemotherapy (standard-therapy group). In the primary intention-to-treat analysis of the first 218 patients, significantly more patients in the IO group achieved CR (80.7% vs 29.4%) and had results below the threshold for minimal residual disease (0.01% marrow blasts) (78.4% vs 28.1%).

In the survival analysis including all 326 patients, progression-free survival was significantly longer with IO (median, 5.0 months vs 1.8 months). While overall survival was only marginally longer, more patients in the IO group underwent allo-HSCT, a subgroup of whom experienced prolonged LFS. Clinically relevant nonhematologic adverse events with IO were hepatotoxicity, with a significantly higher rate of veno-occlusive disease compared with the standard therapy group (11% vs 1%). Sinusoidal obstruction syndrome was most conspicuous among patients who proceeded to HSCT, with a dual-alkylator conditioning regimen constituting a significant risk factor. In a post-hoc analysis to evaluate IO efficacy and safety in older patients vs younger patients treated in the randomized INO-VATE trial, CR/ CRi rates with IO were similar in patients aged ≥ 55 years and patients aged < 55 years (70% vs 75%, respectively). Among IO responders, the MRD-negativity rate was similar among older and younger patients (79% and 76%, respectively). A pediatric trial with inotuzumab in relapsed/refractory B-lineage ALL has started recruitment.

Immunotherapy with bispecific T-cell-engaging antibody

In the development of new monoclonal antibody constructs for B-ALL, the bispecific T-cell engager blinatumomab has showed promising results in relapsed and refractory cases of CD19-positive B-precursor ALL and is approved for that indication. In a multicenter trial for 189

relapsed/refractory BCP-ALL, the composite CR rate was 43%, with a median OS of 6.9 months. Very similar results were obtained in the randomized TOWER study of 405 patients with recurrent or refractory Ph-negative ALL who were randomized to receive blinatumomab or standard chemotherapy, with CR rates of 44% vs 25% and median OS of 7.7 months vs 4 months. Predictors of response, including lower bone-marrow blast-cell counts, extramedullary disease, a high frequency of circulating inhibitory regulatory T cells (Tregs), and expression of PD-L1 on B-cell blasts, have been associated with a poor response.

Single-agent blinatumomab showed comparable antileukemia activity in a phase 2 trial of patients with Ph⁺ ALL who had relapsed or were refractory to TKIs. During the first two cycles, 36% of patients achieved CR/CRh, including four of 10 patients with the T315I mutation. Median relapse-free survival and overall survival times were 6.7 and 7.1 months, respectively.

In a pediatric phase 1-2 trial that treated 93 patients with relapsed/refractory B-lineage ALL, 70 were evaluable after treatment at the finally recommended dose-level. Thirty-nine percent of heavily pretreated patients achieved CR and half were MRD-negative. About a third of the patients could go on to HSCT.

In view of the short response duration and OS, it is recommended that patients achieving a CR proceed to HSCT as soon as possible, using blinatumomab as a bridge to transplantation. Efforts to improve the results of blinatumomab treatment prompted earlier administration in patients still in CR but with detectable MRD. In a recent update, 78% of patients achieved a complete MRD, most after the first treatment cycle. Relapse-free survival (RFS) at 18 months was 53%, and median overall survival was 36.5 months. Complete MRD response was associated with a significantly longer relapse-free survival (23.6 vs 5.7 months); overall survival (38.9 vs 12.5 months) compared with MRD nonresponders. Estimates of relapse-free survival at 18 months were similar with or without censoring for post-blinatumomab HSCT and chemotherapy.

Additional trials moving blinatumomab further forward to frontline therapy are ongoing and in preparation. As with other T-cell therapies, strict attention has to be paid to a set of unique toxicities including neurotoxicity (eg, seizures, encephalopathy) and cytokine-release syndrome that requires close monitoring and prompt intervention.

Immunotherapy with chimeric antigen receptors (CAR T cells)

Targeted immunotherapy using autologous CAR T cells is currently the most potent anti-leukemic modality in the setting of relapsed or refractory ALL. However,

this potency comes at the price of a unique and potentially severe toxicity profile. The most frequently studied transduced constructs contain the variable region of an antibody against the pan B-cell antigen CD19 linked with various costimulatory domains and anchoring part of the T-cell receptor, but CARs, directed against other antigens, are under development and in early phases of clinical development. Such CD19-CARTs have been successfully used to treat patients with multiple relapses of B-lineage (CD19-positive) ALL including relapse after SCT. CR and MRD negativity were achieved in about 70–90% of mostly pediatric patients, resulting in a 1-year overall survival rate of up to 73%. Serious toxicity in the form of cytokine-release syndrome (CRS) and neurotoxicity required intensive care treatment in more than 30% of patients and has caused fatalities in adult patients.

In a recent report of extended follow up of 53 adult patients receiving CD19-CART therapy as salvage therapy for ALL, median event-free- and overall-survival were 6.1 months and 12.9 months, respectively. A low disease burden (<5% bone-marrow blasts) before treatment was associated with enhanced remission duration and survival. In addition, a higher disease burden (≥5% bone marrow blasts or extramedullary disease) had a greater incidence of CRS and neurotoxic events. Control of CRS can be achieved with the anti-IL-6 receptor antibody, tocilizumab. Patients with persistent CAR T-cells remain B-cell depleted and usually receive immunoglobulin substitution. Relapses after CAR therapy have been associated with the loss of persisting circulating CAR T cells in the patient or loss of CD19 on the leukemic cells. To circumvent the latter cause of resistance, dual CARTs, targeting two antigens simultaneously, for example, CD19 and CD22, are being developed. Toxicity and persistence may be partly addressed by construct design, optimization of lymphodepletion therapy before the CAR-T administration, and better characterization of the CAR-T cell product. Ongoing studies seek to define the role of this new technology for more widespread clinical use.

Supportive care and early and late complications of therapy

Initial management

Optimal management of patients with ALL requires careful attention to supportive care because this will impact treatment results. Hyperuricemia and hyperphosphatemia with secondary hypocalcemia are frequently encountered at diagnosis, sometimes even before chemotherapy is initiated, especially in patients with high leukemic cell burden and those with T-cell or mature B-cell ALL. It should be noted

that a large tumor burden per se should not unduly delay the start of chemotherapy. Typically, appropriate measures to counteract tumor-lysis syndrome are administered at the same time as therapy is started. Patients should be hyperhydrated with intravenous fluids (3,000 to 4,500 mL/m²/day) to maintain diuresis and to dilute harmful metabolites. If impaired kidney function is manifested or if the tumor burden is very high and initial treatment has to be rapidly administered to stop progression of the disease, rasburicase (recombinant urate oxidase) should be given to patients at high risk of tumor-lysis syndrome to treat or prevent hyperuricemia; allopurinol may be sufficient if urate concentration is moderately elevated and the risk of tumor lysis is lower. With rasburicase available, there is no indication for alkalinization of urine. A phosphate binder, such as aluminum hydroxide, lanthanum carbonate, or sevelamer, should be given to treat or prevent hyperphosphatemia. Calcium acetate or calcium carbonate may be used if the serum calcium concentration is low, but such treatment is seldom necessary if no alkali is administered.

Infection and antimicrobial prophylaxis

Infections are common both in patients with newly diagnosed ALL as well as in those who are already receiving therapy. During induction as well as during continued therapy, infectious complications can be fatal. Therefore, any patient with ALL who presents with fever, especially those with neutropenia, should be given broad-spectrum antibiotics until infection is excluded. Usually, all patients with ALL are given either trimethoprim-sulfamethoxazole, atovaquone, dapsone, or inhaled pentamidine as prophylactic therapy for *Pneumocystis jirovecii* pneumonia. Some pediatric and many adult trials also recommend some form of antibacterial, antiviral, and antifungal prophylaxis in patients with severe leukopenia during the intensive phases of treatment. The use of high-dose corticosteroids, in particular prolonged dexamethasone, predisposes patients to septicemia and fungal infections. The incidence of different types of infection may differ by center so prophylaxis practices are not uniform. The importance of prevention or early treatment is not only related to the threat of the infection itself but also to the detrimental effect of delaying antileukemic therapy.

The use of hematopoietic growth factors for adults with ALL has been found to be safe and, in some studies, has reduced the number of induction deaths. In pediatric patients, growth factor use is generally limited to situations involving serious post-induction infections or, in some protocols, routinely after the highest intensity block treatments. All blood products should be irradiated prior to SCT to prevent alloimmunization. Other important sup-

portive care measures include the use of indwelling catheters, amelioration of nausea and vomiting, pain control, and continuous psychosocial support for the patient and the family.

Toxic complications during therapy

With improving long-term survival in ALL, the focus on toxicity has increased. Toxicity-reporting is problematic and has not been uniform across protocols. Recently, consensus-definitions across pediatric study groups were described for 14 common toxicities: hypersensitivity to asparaginase, hyperlipidemia, osteonecrosis, asparaginase-associated pancreatitis, arterial hypertension, posterior-reversible encephalopathy syndrome, seizures, depressed level of consciousness, methotrexate-related stroke-like syndrome, peripheral neuropathy, high-dose methotrexate-related severe nephrotoxicity, sinusoidal obstruction syndrome, thromboembolism, and *P jirovecii* pneumonia infection.

Asparaginase is considered an essential chemotherapeutic drug in many protocols but requires attention with respect to toxicity management, particularly of pancreatitis and thrombotic events linked to deranged coagulation parameters. Abdominal pain or pronounced discomfort after asparaginase should prompt consideration of pancreatitis and testing of lipase and amylase, followed by imaging studies if these enzyme levels are clearly elevated.

Coagulation disorders, mostly attributed to asparaginase, are more frequent and clinically threatening in adults than in pediatric patients because these disorders may lead to sinus vein thrombosis, portal vein thrombosis, or other thromboembolic complications. Vigilance is thus necessary, but clinical practice varies, and there is no consensus on preventive measures.

Late effects

Patients who experience many of these acute toxicities will have long-term side effects, for instance, osteonecrosis, associated with high doses of glucocorticoids. Longer continuous use of dexamethasone especially may lead to permanent joint damage and the need for arthroplasty. A recent study indicates that extended use of asparaginase may also enhance this necrotic effect. Acute asparaginase-associated pancreatitis can also cause long-term effects, such as insulin-dependent diabetes, pseudocysts of the pancreas, and exocrine pancreatic insufficiency, in about a third of the acute cases. Several of these toxicities affect central and peripheral nervous function and, even if many of these are usually transient, sequelae with permanent focal deficits, as well as cognitive impairment, may remain.

Other effects may not be noticed following an acute complication. High cumulative doses of steroids also result

in a significantly increased incidence of osteoporosis, which may affect management. It is important to identify any osteoporosis early so that therapeutic intervention and advice on physical exercise to prevent fractures can be implemented. Treatment with anthracyclines, particularly high cumulative doses, can produce severe cardiomyopathy, which may be persistent and progressive years after anthracycline therapy. In current clinical trials, only limited doses of anthracyclines are used, even for high-risk cases, to decrease the risk of subsequent cardiomyopathy; anthracycline-free regimens have also been tried for lower-risk patients.

Cranial irradiation has been implicated as the cause of numerous late sequelae in children and is one of the main reasons to reduce or omit this modality, particularly for younger children. Late effects include second cancers, neurocognitive deficits, and endocrine abnormalities that can lead to obesity, short stature, precocious puberty, and osteoporosis. In general, these complications are seen in girls more often than in boys, and in young children more often than in older children. A long-term follow-up study of survivors of childhood ALL revealed a >10% cumulative risk of second neoplasms at 30 years and a higher than average mortality rate among patients who had received cranial irradiation. The most devastating complication is the development of malignant brain tumors. The median time to the diagnosis of secondary high-grade brain tumor is 9 years, and the median time to diagnosis of meningioma is 20 years. Although neurocognitive problems are linked to cranial irradiation, they also can be caused by systemic and intrathecal therapy. However, irradiation-induced damage has generally had more pronounced effects in comparative studies. Knowledge of potential treatment sequelae that allows modification of treatment strategy and of appropriate screening measures to permit early detection of complications should greatly improve the quality of life of survivors of ALL.

Treatment of Burkitt lymphoma/leukemia in children and adults

Sporadic Burkitt lymphoma (BL) is a rare and highly aggressive B-cell malignancy often presenting with bulky extranodal disease, bone-marrow infiltration, and central nervous system involvement. Tumor growth is extremely rapid, necessitating prompt diagnosis and initiation of treatment. The outcome for both children and adults with Burkitt lymphoma/leukemia has improved dramatically during the past decades. The improved outcomes have resulted from the use of fractionated high doses of alkylat-

ing agents, such as cyclophosphamide or ifosfamide, with high-dose methotrexate. These agents are combined with vincristine, an anthracycline (doxorubicin or daunorubicin), and high-dose cytarabine and administered in rapid succession over 4 to 6 months. Serious toxicity, including infectious complications, nephrotoxicity, or hepatotoxicity, is frequent, but treatment mortality is low. To reduce the large tumor bulk often present at diagnosis and to limit the severity of tumor lysis syndrome, a reduction phase, consisting of a week of glucocorticoid treatment and a dose of vincristine and cyclophosphamide before intensive chemotherapy, has often been incorporated into treatment regimens. Because of an extremely high predisposition to CNS involvement with Burkitt lymphoma/leukemia, intensive CNS-directed therapy is given with high doses of systemically administered cytarabine and methotrexate as well as intrathecal administration with these agents in combination with hydrocortisone. CNS irradiation is typically omitted and is reserved for adult patients with overt CNS disease. Recurrence after the first year rarely, if ever, occurs; therefore, maintenance (continuation) therapy has not been shown to be beneficial and is not recommended. Using this aggressive approach, the survival rate for these patients has ranged from 50% to 60% in many adult series to >80% in pediatric series. Because the lymphoblasts in mature B-cell ALL exhibit strong expression of CD20, several studies have incorporated the anti-CD20 monoclonal antibody rituximab into frontline regimens in an attempt to further improve outcome. Evidence that addition of rituximab to a short intensive chemotherapy program improves EFS in adults with Burkitt's leukemia or lymphoma was demonstrated by a recent randomized phase 3 trial of 260 adult patients with untreated HIV-negative BL who received chemotherapy (lymphome malin B) with or without rituximab (375 mg/m^2) on day 1 and day 6 during the first two courses of chemotherapy (a total of four infusions). Three-year EFS was significantly better in the rituximab group (75% vs 62%). The addition of rituximab to frontline therapies for Burkitt lymphoma/leukemia has also been tested, with promising results, in children in a German pilot study as well as in a smaller nonrandomized cohort from the Children's Oncology Group. An interim analysis from a randomized comparison in a large American-European-Australian consortium, using the LMB backbone including 310 patients, was presented at ASCO 2016. The analysis also indicated a benefit for the rituximab arm (1-year EFS 94 vs 82%). Final results are pending, and the extent of rituximab use in pediatric patients is further tested prospectively in ongoing studies.

If relapse does occur in BL, patients are essentially not salvageable and median overall survival is 3 to 4 months. Efforts to improve therapy by identifying new targetable signaling pathways by comparative genomic analysis have demonstrated a significant association with toll-like receptor (TOLL) signalling, Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signalling ($P < .01$), and mitogen-activated protein kinase (MAPK) signalling ($P < .01$). Within each of these pathways, several kinases were overexpressed, including TLR7, IRAK1, IL-10 receptor, IL-21 receptor PIM1, TYK2 and MAP2K1. Before these treatments can be implemented in the clinic, compounds targeting these pathways will have to be tested as additions to frontline therapy because they are unlikely to be sufficiently effective in the setting of relapsed or refractory disease.

Lymphoblastic lymphoma

Clinical presentation

Lymphoblastic lymphoma is an aggressive neoplasm of T- and B-cell progenitors that represents ~2% to 3% of adult and pediatric nonHodgkin lymphomas. Lymphoblastic lymphoma shares many features of ALL but is arbitrarily distinguished by bone marrow involvement of less than 25%. A precursor T-cell immunophenotype accounts for more than 90% of cases. Thus B-LBL is exceedingly rare. The immunophenotype of T-cell lymphoblastic lymphoma overlaps that of T-cell ALL although the antigen expression profiles may more closely resemble those of late-stage intrathymic T cells than those seen in T-ALL.

A large mediastinal mass is a typical clinical finding in about 90% of cases of T-LBL but not in B-LBL. It may constitute a hematological emergency with superior–vena-cava syndrome, upper airway obstruction, and pericardial or pleural effusions which may be accessed for immunophenotyping. Other frequently involved sites include lymph nodes, skin, bone, gonads, liver, and spleen. CNS disease is more frequently found in patients with bone-marrow involvement and may be a site of relapse. Low-level bone-marrow involvement (minimal disseminated disease, MDD) has been associated with a worse prognosis in some pediatric studies.

Molecular markers

A high frequency of mutations of *NOTCH1* and *FBXW7* genes was found in pediatric T-LBL and was suggested to be a genetic prognostic indicator for T-LBL. Superior survival associated with mutated *NOTCH1/FBXW7* was seen particularly in the absence of *RAS* or *PTEN* abnormalities. A 4-gene oncogenetic classifier based on *NOTCH1/FBXW7*

mutations and *RAS* or *PTEN* alterations was found to be an independent prognostic indicator in adult T-LBL in the GRAALL-LYSA LL03 study.

Therapy

Whereas staging by computed tomography (CT) and positron-emission tomography (PET) is used to confirm initial sites of disease and magnetic resonance imaging (MRI) is employed to assess suspect involvement of bone, brain or heart, initial disease stage does not determine the therapeutic strategy in adults, but CNS-involvement and stage have guided therapy in pediatric patients. Standard treatment is very similar or the same as for ALL, including supportive therapy and special attention to prevention of tumor-lysis syndrome. Because of high rates of mediastinal and CNS relapse, pediatric protocols, in particular, intensified chemotherapy with emphasis on high doses of anti-metabolites. There is no convincing evidence that either allogeneic or autologous SCT is associated with a better outcome than is achieved by intensive chemotherapy. Allogeneic SCT may be considered in high-risk or advanced disease, but patient numbers are too small for any clear recommendation.

CNS-directed therapy

Rigorous CNS prophylaxis is essential and based increasingly on intrathecal chemotherapy to reduce the long-term sequelae of CNS irradiation. Numerous studies have shown that such an approach permits omission of prophylactic cranial irradiation regardless of CNS status at diagnosis. In some clinical protocols, cranial irradiation for advanced CNS disease may still be appropriate.

Radiotherapy to other disease sites

An area of uncertainty in adult patients with T-LBL is the use of mediastinal irradiation (MRT), which has been eliminated from pediatric protocols. It was hoped mediastinal irradiation would reduce the high rate of mediastinal relapse, but this has not been borne out by study data. Higher doses of MRT (36 Gy) have had no benefit in OS, probably because toxicity delayed delivery of chemotherapy. With the success of pediatric-inspired intensive regimens in adult patients, the routine use of MRT no longer appears necessary.

It is controversial whether MRT should be given to adult patients with a residual mediastinal mass. Although PET imaging may more clearly delineate a residual mediastinal mass with viable cells from purely necrotic tissue, it has not been shown to affect survival. Reevaluation by PET or MRI may identify patients requiring supplemental therapy,

including MRT, but this should not delay chemotherapy. The difficulty in identifying those patients for whom MRT is necessary to prevent mediastinal recurrence has not been resolved.

Novel therapies

Immunotherapy and immune-targeted chemotherapy

Bispecific antibodies and CAR-T therapy have been introduced during therapy for relapse. Also these principles are tested using other targets. Targeted chemotherapy (as in previously discussed CD22-directed inotuzumab) is also under development with other specificities. Additionally, naked antibodies directed against CD38, which have been successfully introduced against multiple myeloma, are now planned to be tested in ALL.

The anti-CD30 monoclonal antibody brentuximab vedotin, highly effective in r/r CD30⁺ lymphomas, is conceptually attractive as a treatment for a subset of T-ALL in view of CD30 antigen expression reported in 38% of cases.

Interference with the T-cell checkpoint control system, for instance, by inhibition of PD1 has been successful in some nonhematological malignancies and studies are underway using this principle to enhance the effect of bispecific antibody therapy with blinatumomab.

Pharmacologic inhibition by small molecules

TKIs for *BCR-ABL* positive or *BCR-ABL*-like ALL and JAK-inhibitors for other subtypes with activated kinase profiles have been previously discussed.

An inhibitor of the proteasome system, bortezomib, has been successful in initial trials in relapsed and refractory disease and is now planned to be added to therapy for high-risk patients in induction after relapse in the large international IntReALL study. The related substance carfilzomib, which has lower CNS toxicity, has also shown promise in this context.

Inhibitors of the antiapoptotic BCL-2 family of proteins, of which venetoclax has been approved for r/r chronic lymphocytic leukemia, are mechanistically attractive also in the treatment of ALL. Other principles of interference with antiapoptotic signals, such as MDM-inhibition and mTOR inhibition, are also being explored in early trials as are inhibitors of cell-cycle progression, such as CDK-inhibitors.

The portfolio of novel agents to treat T-ALL has lagged behind those for B-lineage ALL. Among several potentially active small molecules, NOTCH1 inhibitors have attracted the most interest because of the central pathogenic role of activating NOTCH1 mutations in leukemo-

genesis. Clinical results have been mostly disappointing, with management complicated by associated gastrointestinal toxicity and lack of efficacy in relapsed patients with highly proliferative disease. Mitigation of GI toxicity by dexamethasone and testing during earlier stage of disease may enhance the efficacy of this class of drugs.

Based on the high frequency of epigenetic alterations in T-ALL, a number of epigenetic modifying agents have been studied in preclinical models, including DNA methyltransferase inhibitors, HDAC inhibitors, IDH1 and IDH2 mutant inhibitors, BRD4 inhibitors, and DOT1L inhibitors.

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Hodgkin lymphoma

PAMELA B. ALLEN AND ANDREW M. EVENS

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Introduction

Hodgkin lymphoma (HL) represents approximately 10% of all lymphoma cases diagnosed in the United States. This group of diseases usually presents with painless lymphadenopathy involving the neck and chest. Systemic symptoms of fevers, night sweats, and unexplained weight loss may occur in patients with advanced-stage disease (Figure 22-1). Today, the majority of patients with HL are cured with combination chemotherapy with or without radiation. In addition, several novel targeted therapeutic agents have been FDA approved. Given the long-term survival of patients with HL, efforts continue to focus on reducing late, treatment-related toxicities.

Epidemiology

In 2019, approximately 8,500 patients are expected to be diagnosed with HL in the United States, and 1,050 patients are expected to die due to this malignancy. The disease has a bimodal age distribution with one peak in the early 20s and the second in the mid-70s. There is a slight male predominance (male:female incidence ratio of 1.3).

Pathology

HL is a monoclonal lymphoid neoplasm derived from B cells in most cases and is divided into two distinct entities, classical Hodgkin lymphoma (cHL) (95% of cases) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). The malignant cell in cHL, the Hodgkin Reed-Sternberg (HRS) cell, is a large bi-lobed cell with two or more nuclei with eosinophilic nucleoli. HRS cells are derived from germinal center B lymphocytes, but lack a B-cell receptor and several B-cell associated genes and proteins. HRS cells account for the minority of cells in affected lymph nodes and are surrounded by a background of mixed inflammatory cells including B- and T-cells, plasma cells, eosinophils, neutrophils, macrophages, and fibroblasts. In cHL, the HRS express CD30 and CD15. Other B-cell markers are typically reduced or absent including CD20, CD19, and transcription factors OCT-2 and BOB1. PAX-5 also is expressed in HRS cells in most cases. PAX-5 can be helpful in distinguishing cHL from anaplastic large-cell lymphoma, which also expresses CD30 and exhibits large atypical cells but does

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Off-label drug use: Rituximab for the treatment of lymphocyte-predominant Hodgkin lymphoma; HDAC inhibitor and lenalidomide in relapsed/refractory Hodgkin lymphoma.

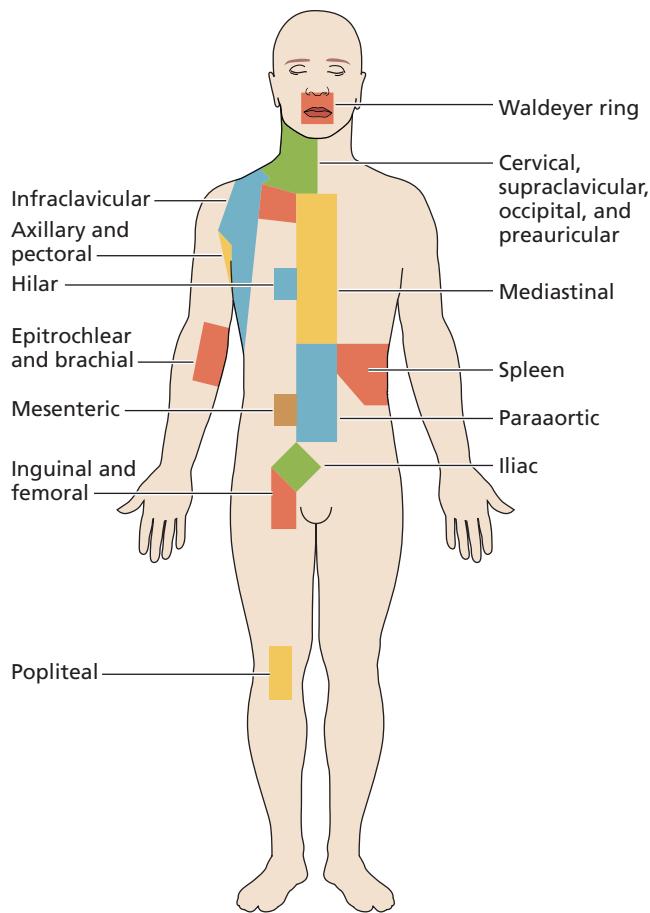


Figure 22-1 Nodal map. The Waldeyer ring includes the pharyngeal tonsil (adenoids), palatine tonsil, and lingual tonsil (base of tongue).

not express PAX-5. Other B- and T-cells markers, including CD45, typically are absent. Epstein-Barr virus (EBV), as evidenced by LMP-1 or EBV small nuclear transcripts (EBER), is found in a subset of cHL, including the majority of cases of mixed cellularity, and nearly all cases of LD HL.

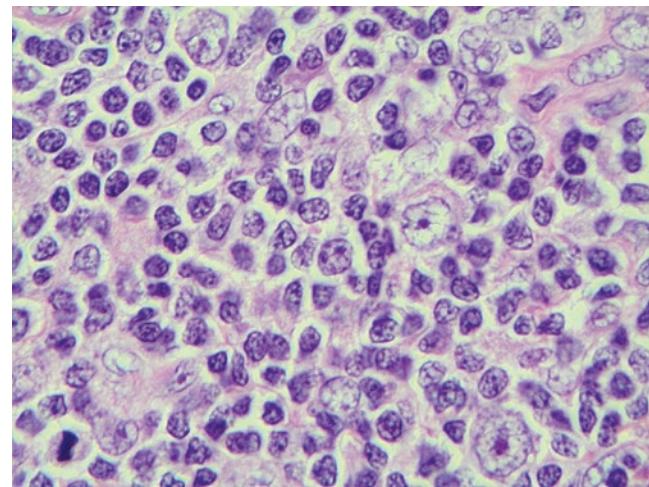
Within classical cHL, there are four histologic subtypes: nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich (LR), and lymphocyte depleted (LD). NS HL is composed of nodular areas with fibrous bands. The HRS cells may be rare in NS but also may be found in sheets (the so-called syncytial variant of NS). In the mixed-cellularity variant, HRS cells are more abundant and are surrounded by neutrophils, eosinophils, macrophages, and plasma cells without areas of fibrosis. The nodal appearance is most commonly diffuse. LR HL typically appears nodular but also can be diffuse. Typical HRS are present in LR HL, and the background is composed predominantly of small lymphocytes. The least common subtype, LD

HL, has a diffuse histologic appearance with a large number (sheets) of HRS cells in a background of fibrosis and necrosis with few inflammatory cells.

NLPHL is morphologically and immunophenotypically distinct from cHL. The “lymphocyte predominant” (LP) cells of NLPHL are “popcorn cells” with lobulated, vesicular nuclei with multiple small nucleoli located peripherally and are found in follicular structures with a partial loss of the B-cell phenotype (Figure 22-2). NLPHL is derived from antigen-selected B cells and expresses typical germinal-center B-markers including BCL-6. Unlike the classic HRS cell, LP cells are typically CD30- and CD15-negative, with CD19-, CD20-, CD45-, and CD79a-positivity (Figure 22-3) and are also PAX-5- and OCT-2-positive. The background lymphocytes are predominantly small CD20⁺ B-cells with rare eosinophils, neutrophils, and plasma cells (Figure 22-4). Surrounding the LP cells are CD4⁺ T-cell rosettes as well as CD21-positive follicular dendritic cells, consistent with the germinal center derivation of this malignancy.

Gray-zone lymphoma (GZL) is an uncommon neoplasm first recognized by WHO as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and cHL. The pathologic diagnosis of GZL is challenging. A spectrum of morphologies with features of cHL and PMBL can occur in GZL, and divergent morphologic areas may be seen within the same tumor specimen. An important morphologic feature of GZL is the abundance of tumor cells, often with confluent sheets of tumor cells. In general, the neoplastic cells in GZL occur in a background containing a paucity of inflammatory cells, although eosinophils, histiocytes, and small lympho-

Figure 22-2 LP or popcorn cells in NLPHL with typical folded, multilobulated nucleus.



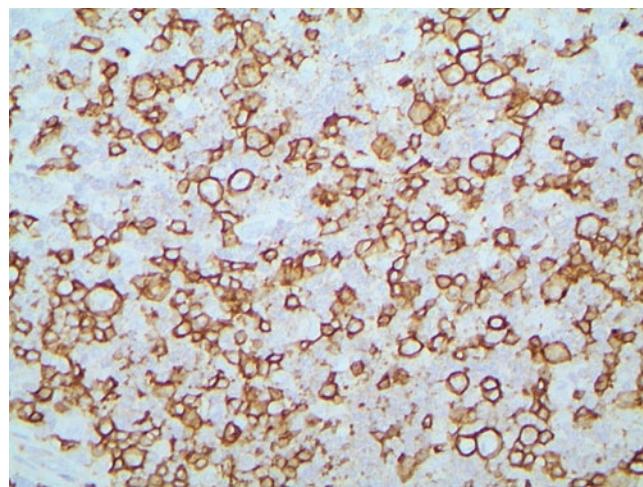


Figure 22-3 CD20 staining on large LP cells in NLPHL.

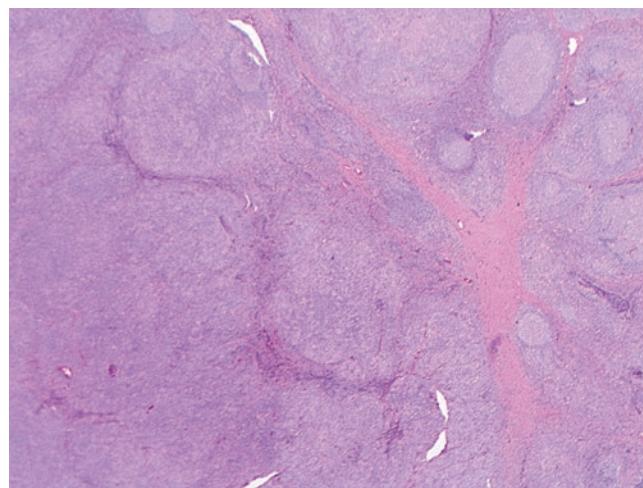


Figure 22-4 Low power view of NLPHL.

cytes can be seen. The immunophenotype is variable with transitional and divergent patterns (ie, tumors with cHL-like morphology can exhibit classic DLBCL or primary mediastinal B-cell lymphoma immunophenotype, and vice versa). In a recent clinicopathologic consensus study, morphology was critical to GZL-consensus diagnosis (eg, tumor cell richness) and immunohistochemistry showed universal B-cell derivation, frequent CD30 expression, and rare EBV positivity.

Pathogenesis

Although HRS cells are derived from germinal center B-cells, HRS cells do not express the majority of germinal-center cell markers and do not transcribe RNA for the production of immunoglobulins or show evidence of somatic hypermutation. NLPHL and cHL are characterized

by constitutive activation of the nuclear factor kappa B (NF- κ B) pathway. cHL also demonstrates increased signaling through the Janus kinase–signal transducer and activation of transcription signaling (JAK-STAT) pathway. HRS utilize immunosuppressive mechanisms to promote survival through programmed cell death 1 (PD-1) signaling as demonstrated by ubiquitous expression of PD ligands 1 and 2 on their cell surface. Genetic analyses have revealed 97% of patients had 9p24.1 alterations, resulting in the up-regulation of the target genes: programmed death 1 ligands (PD-1 ligands) and JAK2. Genetic alterations included 5% polysomy, 56% copy gain, and 36% with amplification. The interaction of PD-1 ligands on the surface of the HRS cell and PD-1 on surrounding T-cells results in down-regulation of T-cell activity and an ineffective immune response against HRS cells. The 9p24.1 amplifications were associated with decreased PFS and more advanced stage in a group of uniformly treated patients with cHL as compared with disomy. Epstein-Barr virus latent membrane protein 1 (LMP1) also induces PD-L1 expression via AP-1 and JAK/STAT pathways, highlighting an additional viral basis for PD-L1 upregulation in EBV-associated cHL.

Risk factors

In developed parts of the Western world the risk of cHL, in particular the NS subtype, is associated with factors indicative of a high standard of living, including small family size, which has been postulated to be related to a delayed exposure to common childhood illnesses or other environmental factors. A diagnosis of infectious mononucleosis confers an increased risk for the subsequent development of cHL. In the developing world and areas of lower socio-economic status, the majority of cases of cHL are of the mixed cellularity and LD subtypes, which are more commonly associated with EBV.

Patients who are immunocompromised, from either human immunodeficiency virus (HIV) infection, immunosuppression due to solid organ or hematopoietic stem cell transplantation (SCT), or who are treated with immunosuppressive medications for autoimmune or inflammatory disease, are at higher risk for the development of cHL, which is typically associated with EBV. The risk of HIV-associated cHL has risen in the era of highly active antiretrovirals. In addition, the risk of cHL is increased in patients with autoimmune diseases, including rheumatoid arthritis, lupus, and sarcoidosis, even in the absence of immunosuppressive therapy.

The risk of developing cHL is higher among relatives of patients with cHL, and specific HLA haplotypes (most notably, HLA-A1) are associated with a higher risk. In identical twins, the risk of HL is increased approximately 100-fold.

KEY POINTS

- Approximately 8,500 new cases of HL are diagnosed per year in the United States.
- cHL is typically CD30-, CD15-, and Pax-5-positive with other negative B-cell markers, whereas NLPHL is CD30- and CD15-negative, with CD19-, CD20-, CD45-, and CD79a- positivity.
- Genetic alterations of 9p24.1 encoding for PDL-1/-2 are present in 97% of cHL cases.

Clinical presentation

Patients with cHL often present with nontender lymphadenopathy. The neck is the most commonly involved site of disease. B symptoms, defined as fevers >100.4°F (38.0°C), drenching night sweats, and involuntary weight loss of >10% of body weight in the preceding 6 months, occur in a proportion of patients with advanced-stage disease but are present in <10% to 20% of patients with early-stage disease. Pruritus, which may be intense and typically is not associated with a rash (although patients may develop secondary excoriations), is seen in 10% to 15% of patients. Although it occurs rarely (<5% of cases), patients may experience intense pain in the sites of disease upon alcohol ingestion.

NS cHL accounts for 70% of cases in the Western world. Males and females are affected in equal proportion and, at diagnosis, most patients are between the ages of 15 and 35 years. Mediastinal involvement, which may be bulky, is more common in NS cHL, and patients may present with respiratory symptoms. Mixed-cellularity cHL is the second most common subtype in the industrial world, representing 20% of cHL. There is a male predominance. Peripheral lymphadenopathy is more common than mediastinal disease, and there is orderly progression from one lymph node basin to the next. LR cHL accounts for 5% of all cases. Patients typically present with early-stage disease affecting peripheral nodes.

LD cHL is the least common subtype at 1% of cases in the Western world. The median age of onset is in the 30s, and males are more often affected. It is more common in the industrial world and in HIV-infected individuals. Extranodal and intra-abdominal disease, advanced-stage disease, and systemic symptoms are common.

There are also racial differences in clinical presentation with Whites presenting at a younger age with NS HL and early-stage disease and Hispanics presenting at older ages with MC HL and advanced-stage disease.

Staging and workup

To make a definite diagnosis of HL, an adequate tissue biopsy is critical. Fine-needle aspirate is not adequate to evaluate architecture and establish the histologic subtype. Incisional or excisional biopsy is preferred, although image-guided core-needle biopsy in patients without peripheral lymphadenopathy may yield sufficient tissue.

Staging should be performed with [¹⁸F]fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) scanning. PET/CT improves the accuracy of staging compared with CT scans alone and is the preferred imaging modality in cHL. Recent studies have demonstrated a high sensitivity of PET/CT for bony involvement. Therefore, bone marrow biopsies are not necessary as part of the initial staging procedures for most patients with cHL younger than 60 years. The Ann Arbor staging system was developed more than 60 years ago with Cotswold modifications for further clarity (Table 22-1). Ann Arbor classification should be used for disease localization; however, patients should be treated as having limited (I, II nonbulky) or extensive (III-IV) disease, with stage II bulky disease generally classified as extensive disease based on the updated 2014 Lugano criteria. Bulky disease is defined as a single nodal mass measuring at least 10 cm in greatest diameter or greater than a third of the transthoracic diameter at any level of thoracic vertebrae as determined by CT. The absence (A) or presence (B) of B symptoms should be recorded.

For restaging using PET/CT, the Deauville 5-point scale (5PS) reading system (Table 22-2) allows for more accurate measurement of response by using a categorical scoring system with a continuous variable. Values are recorded by comparing disease uptake to a reference organ with generally consistent metabolic activity, reducing inter-reader and inter-device inconsistencies.

Initial laboratory assessment should include a complete blood count (CBC) with differential and assessment of renal and hepatic function, including albumin, before initiating chemotherapy. HIV testing should be considered. Erythrocyte sedimentation rate (ESR) commonly is elevated and is prognostic in early stage disease. Lactate dehydrogenase (LDH) is rarely elevated except in patients with extensive, advanced-stage disease. Pulmonary function testing and assessment of cardiac function should be obtained before the initiation of chemotherapy whenever possible but should not delay the initiation of therapy in a young patient without comorbidities.

Table 22-1 Staging of HL

Stage I. Involvement of one lymph node region
Stage II. Involvement of two or more lymph node regions or lymph node structures on the same side of the diaphragm. Hilar nodes should be considered to be "lateralized" and, when involved on both sides, constitute stage II disease. For the purpose of defining the number of anatomic regions, all nodal disease within the mediastinum is considered to be a single lymph node region, and hilar involvement constitutes an additional site of involvement.
Stage III. Involvement of lymph node regions or lymphoid structures on both sides of the diaphragm.
Stage IV. Diffuse or disseminated involvement of one or more extranodal organs or tissue beyond that designated E, with or without associated lymph node involvement.
All cases are subclassified to indicate the absence (A) or presence (B) of the systemic symptoms of significant unexplained fever, drenching night sweats, or unexplained weight loss exceeding 10% of body weight during the 6 months before diagnosis.
The designation "E" refers to extranodal contiguous extension (ie, proximal or contiguous extranodal disease) that can be encompassed within an irradiation field appropriate for nodal disease of the same anatomic extent. More extensive extranodal disease is designated stage IV.
The subscript "X" is used if bulky disease is present. This is defined as a mediastinal mass with a maximum width that is equal to or greater than one-third of the internal transverse diameter of the thorax at any level or >10 cm maximum dimension of a nodal mass.

KEY POINTS

- PET/CT scans are recommended for initial and interim staging evaluation.
- Based on the updated Lugano classification, bone marrow biopsies are not recommended for initial staging in most patients.
- PET scans should be scored utilizing the Deauville 5-point scale (5PS) reading system to limit interreader variability.

Table 22-2 Deauville 5-point scale criteria for evaluation of restaging PET

Score	Criterion
1	No uptake
2	Uptake ≤ mediastinum
3*	Uptake > mediastinum but ≤ liver
4	Moderately increased uptake > liver
5	Markedly increased uptake > liver

*Most common negative vs positive cutoff in clinical trials for early-stage HL (scores 1-2 vs 3-5). Most common negative vs positive cutoff in clinical trials for advanced-stage HL (score 1-3 vs 4-5).

Frontline therapy for early-stage HL

CLINICAL CASE

A 24-year-old woman presents with a persistent dry cough of 2 months duration. She has no weight loss, fever, or night sweats. A chest x-ray reveals a widened mediastinum, and a subsequent chest CT is notable for a 3.5-cm anterior mediastinal mass. Mediastinoscopy and biopsy are performed and reveal classical HL, NS subtype with neoplastic HRS cells expressing CD30, CD15, and negative for CD20. EBER is negative. PET and CT scans demonstrate disease localized to the mediastinum and bilateral hilum. Laboratory studies show a mild leukocytosis at 12.5 with 80% neutrophils and 10% lymphocytes with an otherwise normal CBC. ESR is 25. PET and CT scans after two cycles of therapy show mediastinal uptake less than blood pool, and she completes four cycles of doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD) chemotherapy followed by involved-site radiotherapy (ISRT) to 30 Gy.

Overall, the prognosis of early stage cHL, using currently available therapies, is excellent, with >85% of patients being cured with initial therapy and >95% of patients alive at 5 years. There remains debate regarding use of combined modality therapy (CMT) (ie, chemotherapy followed by consolidative radiotherapy) vs utilizing chemotherapy alone. The more common therapeutic recommendation in Europe includes combined modality therapy given its superior freedom from treatment failure (FFTF). There has been increasing use of chemotherapy alone in North America for HL patients with early-stage disease. Chemotherapy alone is associated with a higher risk of relapse (4% to 8%) but likely has less long-term toxicity compared with combined modality treatment. OS has not been shown to be different, in part, given the salvageability with effective subsequent therapies.

ABVD is the favored chemotherapy for HL in most centers in terms of efficacy and toxicity, including risk of secondary malignancies and infertility. The majority of patients receiving ABVD will develop significant

granulocytopenia. Despite this, retrospective data suggest the risk of febrile neutropenia is quite low, <1% per cycle. Additional retrospective data suggest that receipt of granulocyte growth factors may increase the risk of bleomycin lung toxicity. The majority of HL patients may be treated safely with full-dose therapy, on time, without growth factors. For patients who develop febrile neutropenia, granulocyte colony-stimulating factor (G-CSF) should be administered for the minimal number of days to support the white blood cell count.

Bleomycin-associated pneumonitis is seen in 1% to 3% of patients overall and up to 20% to 30% of patients receiving ABVD older than 60–65 years old, with compromised renal function being a prominent risk factor. There are not well-studied guidelines for prospective monitoring of patients receiving bleomycin. Baseline pulmonary function tests may be obtained before chemotherapy. A low baseline diffusing capacity (DLCO) should be corrected for baseline hemoglobin levels and interpreted carefully in patients with extensive disease in the chest. Collectively, a high index of suspicion is *critical* for the early recognition of bleomycin lung toxicity. Patients who develop cough and/or dyspnea on exertion with or without fevers should be evaluated promptly by physical examination with ambulation and/or at rest for the presence of basilar crackles and oxygen desaturation. Chest x-ray may reveal an interstitial pattern of abnormality, and a decline in the DLCO on pulmonary function testing is typical. Bleomycin should be discontinued *promptly* and steroids should be administered for patients with significant symptoms or hypoxemia. The value of serial pulmonary function testing has not been demonstrated clearly but may show asymptomatic decreases in DLCO.

Radiation therapy

Over time, the extent and dose of radiotherapy (RT) has decreased given associated long-term toxicities, particularly secondary malignancies and cardiac dysfunction, and the improvement in outcomes with the addition of effective chemotherapy. By definition, extended field radiotherapy (EFRT), also known as subtotal nodal radiotherapy (STNRT), includes both the involved lymph nodes and the grossly normal adjacent lymph nodes. Typical extended fields were the mantle field and the inverted Y field. IFRT, which encompassed only the clinically involved lymph nodes, replaced EFRT, in part, based on results of two randomized studies. Current studies are under way to evaluate the use of involved-node radiotherapy (INRT) or involved-site radiotherapy (ISRT), in which 3D planning is used and the initially involved lymph nodes plus an additional margin of 1–5 cm of surrounding, radiographically-uninvolved

tissue are treated. Given that INRT requires high quality pretreatment imaging fused with end-of-treatment scans, most patients receive ISRT.

The dose of RT is dependent in part on the stage and risk of early-stage disease (ie, favorable vs unfavorable), while most recent studies have used 20 to 30 Gy administered in 1.8- to 2-Gy fractions. The German Hodgkin Study Group (GHSG) analyzed two studies in which EFRT at 20, 30, or 40 Gy was administered following COPP/ABVD chemotherapy and demonstrated no difference in OS. Current studies typically employ 20 to 30 Gy of ISRT for nonbulky disease and 30 to 36 Gy of ISRT in the presence of bulk. Although the use of more limited RT fields and lower radiation dose will likely reduce late toxicity compared with that of larger fields, long-term follow-up will be required to confirm this.

Risk stratification

A number of prognostic indicators have been identified in early-stage cHL and are employed in clinical trials to risk stratify patients (Table 22-3). The GHSG scale includes five risk factors, (i) bulky mediastinal disease as defined by more than one-third of the maximal intrathoracic cavity, (ii) ESR of 30 in the presence of B symptoms or (iii) ≥50 without B symptoms, (iv) extranodal extension of disease, and (v) 3 or more lymph node sites of involvement. The EORTC scale includes (i) age ≥50 years, (ii) bulky mediastinal disease, (iii) ESR of 30 in the presence of B symptoms or (iv) ≥50 without B symptoms, and (v) 4 or more nodal sites of involvement. In Canadian and some US cooperative group stud-

Table 22-3 Risk factors in early-stage Hodgkin lymphoma*

Organization	Risk factors
EORTC	Age <50
	No LMA (less than one-third maximum intrathoracic diameter)
	ESR <50 without B sx
	ESR <30 with B sx
	≤ 3 lymph node groups
GHSG	No LMA (less than one-third maximum intrathoracic diameter)
	ESR <50 without B sx
	ESR <30 with B sx
	No extranodal extension
	≤ 2 lymph node groups

*It is important to highlight that the “nodal maps” differ based on GHSG vs EORTC studies.

B sx, fevers, drenching night sweats, unexplained weight loss; EORTC, European Organization for Research and Treatment of Cancer; ESR, erythrocyte sedimentation rate; GHSG, German Hodgkin Study Group; LMA, large mediastinal mass.

Table 22-4 Delineations of lymph node regions by study group

Nodal location(s)	Ann Arbor	GHSG	EORTC
L Cerv, SupraClav, Occip, PreAuric			
L InfraClav, Pec			
L Axilla			
R Cerv, SupraClav, Occip, PreAuric			
R InfraClav, Pec			
R Axilla			
Mediastinum			
L Hilum			
R Hilum			
Total no. of regions	9	5	5

Abbreviations: GHSG, German Hodgkin Study Group; EORTC, European Organisation for Research and Treatment of Cancer; L, left; R, right; Cerv, cervical; SupraClav, supraclavicular; Occip, occipital; PreAuric, preauricular; InfraClav, infraclavicular; Pec, pectoral.

ies, patients with stage IIB disease are considered to have advanced-stage disease. The presence of bulky mediastinal disease is considered to be unfavorable by all groups.

It is important to highlight that the “nodal maps” differ based on GHSG vs EORTC clinical studies (Figure 22-1). In the example given before (ie, bilateral cervical with right-sided supraclavicular and infraclavicular disease), this patient would be classified as early-stage favorable disease in *both* the EORTC and GHSG (Table 22-4).

Early favorable disease

The EORTC H8F and GHSG HD10 established combined modality therapy as a standard of care in early favorable HL. The GHSG HD10 study randomized 1,370 patients without risk factors to combined modality therapy with 4 vs 2 cycles of ABVD with 30 Gy vs 20 Gy of IFRT. With a median follow-up of 7.5 years, there was no difference in FFTF or OS at 5 years in any of the 4 groups, and toxicity was comparable between all arms. The authors concluded that ABVD for 2 cycles followed by 20 Gy IFRT was standard therapy for early-stage favorable HL.

In the follow-up HD13, the GHSG examined the relevance of bleomycin and dacarbazine in the ABVD regimen in patients with favorable-risk early-stage disease. Patients were randomized to 2 cycles of ABVD, omission of bleomycin, dacarbazine, or both followed by 30 Gy of IFRT. The AV and ABV arms were closed early due to adverse events. The 5-year FFTF was 93% in ABVD vs 81%, 89%, and 77%, respectively, in the ABV, AVD, and AV arms. ABVD remained the standard, with AVD showing the least reduction in efficacy of the 3 remaining arms.

The Stanford V regimen, which includes meclorethamine, doxorubicin, vinblastine, prednisone, vincristine, bleomycin, and etoposide, may be used in early-stage favorable or cHL. In patients with stage I or IIA nonbulky cHL, 8 weeks of Stanford V plus 30 Gy of IFRT resulted in an 8-year FFP of 96%. See Table 22-5 for a summary of randomized trials in early favorable HL.

Early unfavorable (intermediate) disease

In patients with unfavorable-risk early-stage HL, combination modality therapy with IFRT over EFRT was established with the EORTC H8U and GHSG HD8 studies. In the GHSG follow-up study, HD11, 4 cycles of ABVD were compared with 4 cycles of BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) and IFRT of 20 or 30 Gy. There were no differences between the chemotherapy arms, so the less toxic ABVD combined with 30 Gy is more often used in this setting.

The subsequent HD14 study examined the role of incorporating initial treatment with escalated BEACOPP followed by ABVD to maximize efficacy and reduce treatment-related toxicity. The rationale was based on data in the advanced setting demonstrating initial treatment with BEACOPP, which resulted in superior PFS compared to ABVD (see discussion on advanced-stage disease). Patients were randomized to standard therapy with 4 cycles of ABVD vs 2 cycles of escalated BEACOPP followed by 2 cycles of ABVD (2+2). All patients received 30 Gy of IFRT. The 2+2 arm resulted in improvement in the primary endpoint of 5-year FFTF at 95% vs 88% ($P < 0.001$) in the standard arm. The OS, however, in both arms was excellent at 97%, highlighting the ability to salvage patients initially treated with ABVD. Grade 3 toxicity was significantly more prominent in the BEACOPP arm in terms of leukopenia, thrombocytopenia, and infection. Occurrence of second malignancies was similar in both arms with two cases of MDS or AML in the BEACOPP group, although the median follow-up remains relatively short at 43 months. See Table 22-6 for a summary of randomized trials in early unfavorable HL.

Chemotherapy alone and PET-adapted therapy

Given the late effects of RT, including secondary malignancies, especially breast cancer in women younger than 30 years and cardiovascular disease, a handful of studies have evaluated the use of chemotherapy only in Hodgkin lymphoma. The Canadian/ECOG HD.6 trial evaluated the use of chemotherapy alone with STNRT without chemotherapy in favorable-risk patients, and CMT with chemotherapy alone in unfavorable-risk patients. The study was closed

Table 22-5 Favorable early stage I-II Hodgkin lymphoma: recent randomized studies*

Trial	No. of patients	Treatment regimens	Outcome	
GHSG HD7 (Engert et al, 2007)	650	EFRT (30 Gy) + IFRT (10 Gy) 2 ABVD + same RT	<u>7-yr FFTF</u> 67% 88% <i>P</i> <.0001	<u>7-yr OS</u> 94% 92% <i>P</i> =.43
EORTC H8F (Ferme et al, 2007)	542	3 MOPP/ABV + IFRT (36 Gy) STLI	<u>5-yr EFS</u> 98% 74% <i>P</i> <.001	<u>10-yr OS</u> 97% 92% <i>P</i> =.001
EORTC H9F (Noordijk et al, 2005)	783	6 EBVP + IFRT (36 Gy) 6 EBVP + IFRT (20 Gy) 6 EBVP (no RT)	<u>4-yr EFS</u> 88% 85% 69% <i>P</i> <.001	<u>4-yr OS</u> 98% 100% 98% <i>P</i> =.241 “No RT” arm closed due to elevated relapse rate
GHSG HD10 (Engert et al, 2005)	1,370	2 ABVD + IFRT (30 Gy) 2 ABVD + IFRT (20 Gy) 4 ABVD + IFRT (30 Gy) 4 ABVD + IFRT (20 Gy)	Median follow-up 53 months, no survival differences between number of ABVD cycles or radiation dose (FFTF 91%-92%, OS 96%-97%)	
NCI-C/ECOG HD.6 (Meyer et al, 2005)	123	ABVD 4-6 cycles STLI	<u>5-yr EFS</u> 87% 88% <i>P</i> =NS	<u>5-yr OS</u> 97% 100% <i>P</i> =NS
GHSG HD13 (Behringer et al, 2015)	1,502	2 ABVD + 30 Gy IFRT 2 ABV + 30 Gy IFRT 2 AVD + 30 Gy IFRT 2 AV + 30 Gy IFRT	<u>5-yr FFTF</u> 93.1% 81.4% 89.2% 77.1% <i>P</i> =NS HR 1.5 for AVD vs ABVD (95% CI-1.00-2.26) AV and ABV arms closed early due to elevated relapse rate	<u>5-yr OS</u> 97.6% 94.1% 97.6% 98.1%

*See text for definitions of favorable early stage category. Minimum HL favorable early stage study size 120 patients.

Abbreviations: EORTC, European Organisation for Research and Treatment of Cancer; EBVP, epirubicin, bleomycin, vinblastine, prednisone; IFRT, involved-field radiation therapy; EFS, event-free survival; OS, overall survival; GHSG, German Hodgkin Study Group; FFTF, freedom from treatment failure; ABVD, doxorubicin, vinblastine, bleomycin, dacarbazine; FFP, freedom from progression; STLI, subtotal nodal irradiation; NS, not significant; RT, radiotherapy.

early after the EORTC study demonstrated the superiority of CMT using IFRT compared with STNRT. However, with extended follow-up, treatment with chemotherapy alone was found to be noninferior owing to excess mortality from other causes in the radiation-containing arm. At a median follow-up of 11.3 years, there was no difference in the freedom-from-progressive disease rates at 89% and 87%, respectively, or OS at 98% in both arms. In contrast, Hay et al found that those patients who were not in CR by CT scan after two cycles of ABVD had a significantly poorer outcome. Chemotherapy-alone approaches were also assessed by PET-directed approaches in both the RAPID-UK and EORTC H10 studies.

The United Kingdom (UK) RAPID trial assessed PET-directed de-escalation of therapy in patients with stage IA/IIA HL. PET scans were performed after three cycles of ABVD. PET-negative patients (Deauville score of 1 or 2) were randomized to receive no further therapy vs IFRT and PET-positive patients (Deauville score of 3 to 5) received consolidation with IFRT. At a median follow-up of 60 months, 3-year PFS rates for PET-3-negative patients who received CMT vs chemotherapy alone were 94.6% vs 90.8%, respectively, which exceeded the pre-specified noninferiority boundary. Altogether, these data suggested noninferiority was *not present* for 3-year PFS, although outcomes were excellent in both groups. Overall survival at

Table 22-6 Intermediate early-stage I-II Hodgkin lymphoma: randomized chemotherapy studies*

Trial	No. of patients	Treatment regimens	Outcome	
EORTC H6U (Carde et al, 2005)	316	3 MOPP + Mantle + 3 MOPP 3 ABVD + Mantle + 3 ABVD	<u>10-yr FFS</u> 77% 88% <i>P</i> <.0001	<u>10-yr OS</u> 87% 87% <i>P</i> =.52
EORTC H7U (Noordijk et al, 2006)	316	6 EBVP + IFRT (36 Gy) 6 MOPP/ABV + IFRT	<u>6-yr EFS</u> 68% 88% <i>P</i> <.001	<u>6-yr OS</u> 79% 87% <i>P</i> =.0175
GHSG HD11 Eich et al, 2010)	1,395	4 ABVD + IFRT (30 Gy) 4 ABVD + IFRT (20 Gy) 4 BEACOPP-base + IFRT (30 Gy) 4 BEACOPP-base + IFRT (20 Gy)	<u>5-yr FFTF</u> 88.3% 81.1% 87.0% 86.8% <i>P</i> =NS*	<u>5-yr OS</u> 94.3% 93.8% 94.6% 95.1% <i>P</i> =NS
EORTC H8U (Fermé et al, 2007)	996	6 MOPP/ABV + IFRT (36 Gy) 4 MOPP/ABV + IFRT (36 Gy) 4 MOPP/ABV + STLI	<u>5-yr EFS</u> 84% 88% 87% <i>P</i> =NS	<u>5-yr OS</u> 88% 85% 84% <i>P</i> =NS
NCI-C/ECOG (Meyer et al, 2005)	276	ABVD 4-6 cycles ABVD 2 cycles+STLI	<u>5-yr EFS</u> 88% 92% <i>P</i> =.09	<u>5-yr OS</u> 95% 92% <i>P</i> =NS
EORTC H9U (Fermé et al, 2005)	808	6 ABVD + IFRT (30 Gy) 4 ABVD + IFRT (30 Gy) 4 BEACOPP-base + IFRT (30 Gy)	<u>4-yr EFS</u> 91% 87% 90% <i>P</i> =NS	<u>4-yr OS</u> 95% 94% 93% <i>P</i> =NS
GHSG HD14 (Borchmann et al, 2008)	1,216	4 ABVD + IFRT (30 Gy) 2 BEACOPP-esc + 2 ABVD + IFRT (30 Gy)	<u>5-yr FFTF</u> 87.7% 94.8% <i>P</i> <.001	<u>5-yr OS</u> 96.8% 97.2% <i>P</i> =NS

*See text for definitions of intermediate early stage category. Minimum study size 250 patients.

Abbreviations: EORTC, European Organisation for Research and Treatment of Cancer; GELA, Groupe d'Etude des Lymphomes de l'Adulte; GHSG, German Hodgkin Study Group; NCI-C, National Cancer Institute of Canada; EORTC, Eastern Cooperative Oncology Group; MOPP, mechlorethamine, vincristine, procarbazine, prednisone; ABVD, doxorubicin, vinblastine, bleomycin, dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin (Adriamycin), cyclophosphamide, vincristine, procarbazine and prednisone; base, baseline; FFS, failure-free survival; OS, overall survival; IFRT, involved-field radiation therapy; STLI, subtotal nodal irradiation; EFS, event-free survival; FFTF, freedom from treatment failure; RFS, relapse-free survival; NS, not significant.

3 years was 97% in IFRT arm and 99% in the non-IFRT arm, which was nonsignificant.

The H10F and H10U studies, led by the EORTC, randomized favorable (F) and unfavorable (U) early-stage HL patients (using EORTC definitions) to PET-based versus non-PET-based treatment strategies. At preplanned interim analysis, more early progressions were noted in the chemotherapy-only arm than in the CMT arm for both

F and U cohorts. Therefore, the study was amended to add INRT to all treatment arms. In subsequent follow-up of PET-negative patients, 5-year PFS rates in the F group were 99.0% vs 87.1% in favor of ABVD+INRT; the U group, 92.1% vs 89.6 in favor of ABVD+INRT. In the F group CMT resulted in a greater difference in PFS (11.9%) than in the U group (2.5%). The authors concluded that in the unfavorable group chemotherapy alone could be

considered. Another objective of the H10F/U studies was to determine if intensification of therapy from ABVD therapy to escalated BEACOPP could improve outcomes for interim FDG-PET-2 positive patients. Of 1,950 randomly assigned patients, 19% were PET positive. The 5-year PFS improved from 77.4% for standard ABVD+INRT to 90.6% for intensification to BEACOPPesc + INRT (HR 0.42; $P=.002$). See Table 22-7 for a summary of response-adapted trials in early-stage HL.

Summary of frontline therapy for early-stage HL

For patients with favorable disease, current options include 3–4 cycles of ABVD with or without ISRT, typically 30 Gy, except for patients meeting the criteria for the GHSG HD 10 study, where 2 cycles of ABVD plus 20 Gy ISRT is an option. Interim PET scans after 2–3 cycles are standard. Those patients with a CR on interim imaging may be treated with as few as 3–4 cycles of chemotherapy, omitting radiation. This strategy results in a slightly increased risk of HL progression (4% to 6%), though overall survival rates are similar. The Stanford V regimen for 8

weeks plus 30 Gy of IFRT to sites >5 cm is an alternative approach.

For patients with unfavorable, nonbulky disease, options include 4 cycles of ABVD plus 30 Gy of ISRT or escalated BEACOPP x2 followed by ABVD x2 followed by 30 Gy of ISRT in patients fitting the criteria for the GSHG HD14 study. The use of escalated BEACOPP in this setting results in improved disease control at the expense of increased toxicity without an OS benefit. Chemotherapy alone with 6 cycles of ABVD for patients without bulky disease is an alternative, especially in young women desiring to lower the risk of infertility and those at risk for RT-related breast cancer; this strategy results in 5% to 8% lower PFS without a difference in OS.

For early-stage nonbulky patients with PET-2 positivity in the EORTC HD10 study, 5-year PFS was improved 13.2% with intensification to 2 cycles of BEACOPPesc plus 30 Gy INRT (vs continuation of ABVD for 4 to 6 cycles). For patients with bulky disease, options include four to six cycles of ABVD or 12 weeks or Stanford V followed by 36 Gy of IFRT.

Table 22-7 Randomized phase 3 response-adapted studies in adult early-stage (I-II) Hodgkin lymphoma

Trial	Patient group	Enrollment	Results
EORTC/LYSA/FIL H10F	Favorable group	761 patients (381 patients PET negative)	5-year PFS in favorable group 99% (experimental) vs 87% (standard arm) (HR, 15.8; 95% CI, 3.8–66.1)
EORTC/LYSA/FIL H10U	Unfavorable/intermediate group	1,191 patients (519 patients PET negative)	5-year PFS in unfavorable group 92.1% (experimental) vs 89.6% (standard arm) (HR, 1.45; 95% CI, 0.8–2.5)
EORTC/LYSA/FIL H10F/U	Favorable and intermediate groups	361 patients PET positive (BEACOPPesc vs ABVD)	5-year PFS 77% for standard ABVD + INRT vs 91% for intensification to BEACOPPesc + INRT (HR 0.42; 95% CI, 0.23–0.74; $P=.002$)
UK NCRI RAPID	Favorable and unfavorable/intermediate groups combined (nonbulky)	602 patients (PET negative in 75%)	3-yr PFS for no RT vs IFRT in PET neg pts: 91% vs 95% by ITT ($P=.23$) and 91% vs 97% by per protocol analysis ($P=.03$); 3-yr PFS for PET pos: 85%
GHSG HD16 (NCT01356680)	Favorable group	Standard arm: 2×ABVD + 20 Gy IF-RT Experimental arm: 2×ABVD for all patients, subsequent stratification by FDG-PET; for PET-positive patients: + 20 Gy IF-RT; for PET-negative patients: end of treatment	Results pending
GHSG HD17 (NCT00736320)	Unfavorable/intermediate group	Standard arm: 2 cycles BEACOPP escalated + 2 cycles ABVD followed by 30 Gy IFRT irrespective of FDG-PET results Experimental arm: 2 cycles BEACOPP escalated + 2 cycles ABVD followed by 30-Gy INRT if FDG-PET is positive after chemotherapy; 2 cycles BEACOPP escalated + 2 cycles ABVD if FDG-PET is negative after chemotherapy	Results pending

Abbreviations: EORTC, European Organization for Research and Treatment of Cancer; LYSA, Lymphoma Study Association; FIL, Fondazione Italiana Linfomi; UK NCRI, United Kingdom National Cancer Research Institute; GHSG, German Hodgkin Study Group; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval.

KEY POINTS

- Risk factors for early stage HL include the presence of bulky disease, ESR, and number of nodal sites of involvement.
- More than 90% of patients with favorable disease and 85% of patients with unfavorable disease are cured with initial therapy.
- Therapeutic options for favorable early-stage HL by EORTC criteria include 3–4 cycles of ABVD +/- ISRT (30 Gy).
- Patients with favorable disease by GHSG HD10 criteria are eligible for 2 cycles of ABVD + 20 Gy ISRT.
- Therapeutic options for unfavorable early-stage HL include 4 cycles of ABVD + 30 Gy ISRT; 2 cycles of escalated BEACOPP followed by 2 cycles of ABVD + 30 Gy ISRT in patients fitting criteria for the GSHG HD14; or chemotherapy alone with ABVD for 4–6 cycles if interim PET is negative.

chemotherapy, in contrast to early stage cHL, where the long-term cure exceeds 90%. Different prognostic indices are utilized for early- and advanced-stage disease. Prognosis in advanced-stage may be defined by the International Prognostic Index (IPS) (Table 22–8), originally published in 1998, including measurements of albumin, hemoglobin, sex, age older than 45 years, stage IV, and the presence of leukocytosis or lymphocytosis. Patients with an IPS ≥3 were found to have inferior treatment outcomes and were identified as potentially requiring more intensive therapy. In an updated analysis of the IPS performed by the British Columbia Cancer Agency, 5-year FFP ranged from 62% to 88% and 5-year OS ranged from 67% to 98% with narrower ranges of outcomes for patients ages younger than 65 years (FFP ranging from 70% to 88% and 5-year OS ranging from 73% to 98%). Controlling for all IPS factors, only age and hemoglobin level retained independent significance.

Initial treatment options for advanced-stage disease include ABVD, Stanford V, or escalated BEACOPP (Table 22–9). Recently, results of the randomized phase 3 study, ECH-ELON-1, assessing a novel regimen replacing bleomycin in ABVD with the CD-30 drug antibody conjugate, brentuximab vedotin (A+AVD), have been reported. This regimen has challenged the role of ABVD as the standard frontline regimen in this patient population.

Interim PET after 2 cycles of therapy is generally recommended and may allow for further adjustments in therapy depending on response. Gallamini et al evaluated 260 patients with stage IIB-IV HL, most of them treated with ABVD chemotherapy. Patients underwent PET scans after two cycles of therapy but continued with ABVD regardless of the PET result. Approximately 20% of patients were

Frontline therapy for advanced-stage HL

CLINICAL CASE

A 55-year-old man with a history of hypertension and asthma presented with a firm, fixed 3–4 cm right-sided submandibular and cervical adenopathy. Biopsy of a right axillary lymph node demonstrated large, pleomorphic lymphoma cells positive for CD15 and CD30 and negative for ALK-1, CD3, CD20, and CD45, that was consistent with cHL, NS subtype. PET and CT scans demonstrated extensive bilateral cervical, supraclavicular, axillary, mediastinal, hilar, and retroperitoneal adenopathy with standardized uptake values (SUVs) of 7.3–18.5, and small bilateral pulmonary nodules. The patient had no B symptoms, ESR was elevated at 82, and he had six adverse prognostic features by the International Prognostic Score (IPS), including male gender, age >45 years, white blood cells (WBC) 15.5, hemoglobin (Hb) 8.6 mg/dL, albumin 3.0 g/dL, and stage IV disease. Ejection fraction (EF) was normal at 55% on pretreatment multigated acquisition scan. Treatment was given with six cycles of ABVD without complication until 2 days after completion of cycle 6 when the patient noticed progressive dyspnea with exercise. Chest x-ray and a CT scan of the chest demonstrated no pulmonary infiltrates or nodules, but an echocardiogram demonstrated an ejection fraction (EF) of 20%.

Advanced-stage disease is generally classified as Ann Arbor stage III and IV, but clinical trials have often incorporated patients with high-risk stage II disease, such as those with B-symptoms, multiple sites, and/or bulky disease. Approximately 70% to 80% of younger patients with advanced-stage HL remain disease free at 10 years with conventional

Table 22–8 International Prognostic Score in advanced-stage HL

No. of risk factors*	5-year FFP (%)		5-year OS (%)	
	1998	2012	1998	2012
0	84	88	89	98
1	77	85	90	97
2	67	80	81	92
3	60	74	78	91
4	51	68	61	88
>5	42	70	56	73

From Hasenclever D, Diehl V. *N Engl J Med.* 1998;339:1506–1514, and Moccia AA, Donaldson J, Chhanabhai M, et al. *J Clin Oncol.* 2012;30:3383–3388.

FFP, freedom from progression; OS, overall survival.

* The IPS is derived from a retrospective analysis of 5,141 patients treated at 25 centers from 1983–1992 with advanced-stage HL. Risk factors identified in this retrospective study included age >45 years, male gender, WBC >15,000/mm³, Hb <10.5 g/dL, absolute lymphocyte count <600/mm³ or <8% of WBC, albumin <4.0 g/dL, and stage IV disease. More recent data on the value of IPS suggest that the impact might have narrowed in the modern treatment era (Moccia et al, 2012).

Table 22-9 Frontline chemotherapy regimens in HL

Regimen	Drugs	Method of administration	When administered	Cycle
ABVD	Doxorubicin 25 mg/m ²	IV	Days 1 and 15	Q 28 days
	Bleomycin 10 units/m ²	IV	Days 1 and 15	
	Vinblastine 6 mg/m ²	IV	Days 1 and 15	
	Dacarbazine 375 mg/m ²	IV	Days 1 and 15	
BEACOPP (baseline)	Bleomycin 10 mg/m ²	IV	Day 8	Q 21 days
	Etoposide 100 mg/m ²	IV	Days 1–3	
	Doxorubicin 25 mg/m ²	IV	Days 1	
	Cyclophosphamide 650 mg/m ²	IV	Day 1	
	Vincristine 1.4 mg/m ² (capped at 2.0 mg)	IV	Day 8	
	Procarbazine 100 mg/m ²	IV	Days 1–7	
	Prednisone 40 mg/m ²	IV	Days 1–14	
BEACOPP (escalated)	Bleomycin 10 mg/m ²	IV	Day 8	Q 21 days
	Etoposide 200 mg/m ²	IV	Days 1–3	
	Doxorubicin 35 mg/m ²	IV	Days 1	
	Cyclophosphamide 1,250 mg/m ²	IV	Day 1	
	Vincristine 1.4 mg/m ² (capped at 2.0 mg)	IV	Day 8	
	Procarbazine 100 mg/m ²	IV	Days 1–7	
	Prednisone 40 mg/m ²	IV	Days 1–14	
Stanford V	Doxorubicin 25 mg/m ²	IV	Weeks 1, 3, 5, 7, 9, 11	
	Vinblastine 6 mg/m ²	IV	Weeks 1, 3, 5, 7, 9, 11	
	Vincristine 1.4 mg/m ² (capped at 2.0 mg)	IV	Weeks 2, 4, 6, 8, 10, 12	
	Bleomycin 5 U/m ²	IV	Weeks 2, 4, 6, 8, 10, 12	
	Mustard 6 mg/m ²	IV	Weeks 1, 5, 9	
	Etoposide 60 mg/m ²	IV	Weeks 3, 7, 11	
	Prednisone 40 mg/m ²	PO QOD	Weeks 1–9; taper by 10 mg	
			QOD weeks 10 and 11	

IV, intravenous; PO, per os (by mouth); Q, every; QOD, every other day.

PET positive. At a median follow-up of 2 years, the PFS in PET-negative patients was 95%, whereas only 13% of patients with a positive PET scan were free from disease ($P < 0.0001$). The negative predictive value (NPV) of an interim PET scan following ABVD is relatively high, ranging from 86% to 95%, but the positive predictive value (PPV) may be as low as 44%. In contrast, the NPV of escalated BEACOPP is very high, generally estimated at >95%. Given the prognostic value of interim PET, several studies have assessed the value of escalating or de-escalating therapy based on interim PET results.

See Table 22-10 for a summary of randomized trials in advanced HL.

ABVD

Since the early 1990s, the treatment of patients with advanced-stage HL has relied on combination chemotherapy with ABVD (Table 22-9). ABVD is associated with minimal effect on fertility and minimal secondary myelodysplasia or leukemia.

ABVD was superior to older regimens, including the MOPP/ABV hybrid, due to reduced treatment-related toxicity in spite of similar CR and FFS rates. The 5-year OS was 82% using ABVD. In contrast to MOPP/ABV, ABVD therapy resulted in fewer pulmonary and hematologic toxicities, treatment-related deaths, and second malignancies, including acute leukemia. In a UK study comparing ABVD

Table 22-10 Summary of randomized frontline trials in advanced stage Hodgkin lymphoma

Trial	No. of patients	Treatment regimens	Outcome	
Milan (Santoro et al, 1987)	232	ABVD 6 cycles + STLI	<u>7-yr EFS</u>	<u>7-yr OS</u>
		MOPP 6 cycles + STLI	81%	77%
			63%	68%
			<i>P</i> <.002	<i>P</i> <.03
CALGB (Canellos et al, 1992)	361	ABVD 6–8 cycles	<u>5-yr FFS</u>	<u>5-yr OS</u>
		MOPP 6–8 cycles	61%	73%
		MOPP/ABVD 12 cycles	50%	66%
			65%	75%
			<i>P</i> =.03	<i>P</i> =NS
CALGB (Duggan et al, 2003)	856	ABVD 8–10 cycles	<u>5-yr FFS</u>	<u>5-yr OS</u>
		MOPP-ABV 8–10 cycles	63%	82%
			66%	81%
			<i>P</i> =NS	<i>P</i> =NS
GHSG HD9 (Diehl et al, 2003)	1,201	COPP/ABVD×8 cycles + IFRT*	<u>5-yr FFTF</u>	<u>5-yr OS</u>
		BEACOPP-baseline×8 cycles + IFRT*	69%	83%
		BEACOPP-escalated×8 cycles + IFRT*	76%	88%
			87%	91%
			<i>P</i> <.002	<i>P</i> <.002
United Kingdom (Johnson et al, 2005)	807	ABVD 6 cycles + 30–35 Gy*	<u>3-yr EFS</u>	<u>3-yr OS</u>
		MDR regimen (ChlVPP/PABIOE or ChlVPP/EVA) 6 cycles + 30–35 Gy*	75%	90%
			75%	88%
			<i>P</i> =NS	<i>P</i> =NS
GHSG HD12 (Engert et al 2006; Diehl et al, 2008)	1,502	BEACOPP-escalated×8 cycles	<u>4-yr FFTF</u>	<u>4-yr OS</u>
		BEACOPP×4 esc and 4 base cycles	86%	88%
		BEACOPP+30 Gy IFRT	91%	91%
		BEACOPP without RT	91%	95%
			88%	95%
			<i>P</i> =NS	<i>P</i> =NS
Intergruppo Italiano Linfomi (Gobbi et al, 2005)	355	ABVD×6 cycles + IFRT (RT 62% of pts)*	<u>5-yr PFS</u>	<u>5-yr OS</u>
		MOPPEBVCAD×6 cycles + IFRT (RT 66%)*	85%	90%
		Stanford V×3 cycles + IFRT (RT 47%)*	94%	89%
			73%	82%
			<i>P</i> <.01	<i>P</i> <.04
Intergruppo Italiano Linfomi (Federico et al, 2009)	307^	ABVD×6 cycles (RT 46%)	<u>5-yr PFS</u>	<u>5-yr OS</u>
		COPPEBVCAD×6 cycles (RT 43%)	68%	84%
		EscBEACOPP×4, baseBEACOPP×2 (RT 44%)	78%	91%
			81%	92%
			<i>P</i> <.038	<i>P</i> =NS
United Kingdom (Johnson et al, 2008)	520	Stanford V×12 weeks	<u>5-yr PFS</u>	<u>5-yr OS</u>
		ABVD 6–8 cycles	74%	92%
			76%	90%
			<i>P</i> =NS	<i>P</i> =NS

Table continues on next page

Table 22-10 Summary of randomized frontline trials in advanced stage Hodgkin lymphoma (continued)

Trial	No. of patients	Treatment regimens	Outcome	
GHSG HD15 (Engert et al, 2012)	2,126	BEACOPP-esc × 8 cycles +/- 30Gy IFRT*	5-yr FFTF	5-yr OS
		BEACOPP-esc × 6 cycles +/- 30Gy IFRT*	84.4%	91.9%
		BEACOPP-14 × 8 cycles +/- 30Gy IFRT*	89.3%	95.3%
			89.4%	94.5%
			P=.009	P<.019
EORTC 20012 (Carde et al, 2016)	549	For patients IPS 4-7 only: ABVD × 8 cycles	4-yr EFS	486.7-yr OS
		BEACOPP × 4 esc and 4 base cycles	63.7%	69.3%
			69.3%	90.3%
			P=NS	P=NS

Minimum study size 230 patients.

*Radiation delivered to sites of initial bulk disease or partial remission after chemotherapy. For GHSG HD15, radiation was given only to patients with disease >2.5 cm following chemotherapy that was PET positive.

Abbreviations: IFRT, involved-field radiation therapy; STLI, subtotal nodal irradiation; EFS, event-free survival; OS, overall survival; FFS, failure-free survival; FFTF, freedom from treatment failure; MOPP, mechlorethamine, vinorelbine, procarbazine, prednisone; ABVD, doxorubicin, vinblastine, bleomycin, dacarbazine; MDR, multidrug resistant; ChIVPP/PABIOE, chlorambucil, vinblastine, procarbazine, prednisone/prednisolone, doxorubicin, bleomycin, vinorelbine, etoposide; EVA, etoposide, vinorelbine, and doxorubicin; GHSG, German Hodgkin Study Group; BEACOPP, bleomycin, etoposide, doxorubicin (Adriamycin), cyclophosphamide, vinorelbine, procarbazine, prednisone; esc, escalated; base, baseline; MOPPEBVCAD, mechlorethamine, vinorelbine, procarbazine, prednisone, epoxorubicin, bleomycin, vinblastine, lomustine, doxorubicin, and vindesine; IPS, international prognostic score; NS, not significant; RT, radiotherapy.

to other combination hybrid regimens (ChIVPP/PABIOE and ChIVPP/ EVA), the 3-year EFS and OS with ABVD were 75% and 90%, respectively, with less infectious and neurologic toxicity than observed with the hybrid regimens. As a result of these trials, ABVD became the standard of care for initial therapy of advanced-stage HL.

ABVD is routinely followed by interim PET scanning and, more recently, PET results have been used to alter therapy. The largest trial to date assessing PET-directed therapy was the RATHL study. RATHL was a large randomized phase 3 clinical trial led by the United Kingdom. Patients had stage IIB to IV disease or stage IIA disease with adverse features (eg, bulky disease or at least three involved sites). Interim PET was performed after 2 cycles, with negativity defined as Deauville score of 1-3. Patients with a negative PET-2 scan were randomized either to continuation of ABVD or to omission of bleomycin (AVD group) for cycles 3-6. The positive PET group received BEACOPP. Radiotherapy was not recommended for patients with negative findings on interim scans. In those treated with ABVD or AVD, results demonstrated that de-escalation to AVD in patients with a negative PET2 was noninferior, with a 3-year PFS of 85.7% and 84.4% and OS of 97.2% and 97.6%, respectively. Additionally, respiratory adverse events were more severe in the bleomycin-containing group. The PET positive group (n=182) had a 74.4% rate of negative repeat PET after BEACOPP; the 3-year PFS was 67.5% and the 3-year OS was 87.8%. Escalation to BEACOPP in PET2-positive patients was also supported in a large phase 2 study led by the US Intergroup: with a median follow-up of 39.7 months, the 2-year PFS was 82% for PET2-negative and 64% for

PET-2-positive patients who switched to eBEACOPP, compared to the historic control from the Gallamini trial of 13% PFS at 2 years in patients who continued ABVD.

Stanford V

ECOG 2496, a randomized phase 3 US Intergroup trial, demonstrated no significant difference in 5-year FFS (73% vs 71%, P=.29) or 5-year OS (88% vs 87%, P=.87) in 812 patients with bulky stage I-II, III, or IV HL receiving 6-8 cycles of ABVD with radiation to bulky mediastinal disease or 12 weeks of Stanford V chemotherapy with RT to disease >5 cm or splenic nodules, respectively. With median follow-up of 5.25 years, 26 second malignancies were observed (14 in the ABVD group and 12 in Stanford V group). There was more pulmonary toxicity among patients treated with ABVD and more myelosuppression and neuropathy with patients in the Stanford V arm. Overall, Stanford V may be acceptable in selected patients for whom a shortened treatment duration or reduction in cumulative doses of bleomycin or doxorubicin is desirable.

BEACOPP

BEACOPP is a German-derived regimen that has been studied in 3 major varieties: baseline BEACOPP, BEACOPP-14, and escalated BEACOPP. Escalated BEACOPP (escBEACOPP) differs from ABVD by incorporating elevated doses of etoposide, doxorubicin, and cyclophosphamide. Several randomized comparisons of these regimens have identified improved PFS with BEACOPP compared to ABVD (PFS=65% to 70% in ABVD at 10 years compared with 75% to 85% with escBEACOPP) but with similar OS of

approximately 75% to 85% at 10 years (Table 22-9). However, a large meta-analysis comparing initial treatment with ABVD to escBEACOPP demonstrated a significant improvement in overall survival in patients who were treated with 6 cycles of escalated BEACOPP initially, with an absolute OS difference of 5% to 10% at 5 years. Improved disease control comes at the expense of increased rates of infertility, grade-four infections, hospitalizations for neutropenia, and a slightly increased risk of secondary hematologic malignancies compared to ABVD. Also, the GHSG HD9 trial demonstrated no improvement in survival or FFTF and an increased toxicity with escBEACOPP compared with COPP/ABVD in patients aged 60 to 65 years.

Thus, escBEACOPP is *not recommended* for patients older than 60 years. See Table 22-11 for further details.

Four additional large randomized studies (2 French studies and 2 Italian studies), compared ABVD with escBEACOPP followed by BEACOPP baseline. These studies showed superior DFS in the escalated BEACOPP arms without significant improvements in OS. In the Italian and French studies 5-year PFS was 81% to 85% in BEACOPP arms compared to 65% to 73% with ABVD. The EORTC 2012 Intergroup Trial focused on patients with high-risk advanced-stage disease. Patients with stage III/IV disease and an IPS score of ≥ 3 were randomized to 8 cycles of ABVD vs 4 cycles of escBEACOPP plus 4 cycles of baseline

Table 22-11 Response-adapted studies in advanced-stage (III/IV) HL

Trial	Phase	No patients	Treatment	Outcomes
HD0801	2	519 416 103	ABVD $\times 3 \rightarrow$ PET-2 PETneg: ABVD $\times 4$ PETpos: HDCT + SCT	81% 2-year PFS 76% 2-year PFS
SWOG S0816	2	336 271 60	ABVD $\times 2$ PETneg: ABVD $\times 4$ PETpos: escBEACOPP $\times 6$	79% 2-year PFS 64% 2-year PFS
GITIL HD0607	3	782	ABVD $\times 2 \rightarrow$ PET-2 PETneg: ABVD $\times 4$, randomize RT vs no RT PETpos: randomize escBEACOPP vs BEACOPP +/- rituximab	87% 3-year PFS 60% 3-year PFS *No difference based on receipt of radiation for PET2 negativity or rituximab for PET2 positivity
RATHL	3	1412	ABVD $\times 2 \rightarrow$ PET-2 PETneg: ABVD $\times 4$ AVD $\times 4$ PETpos: escBEACOPP BEACOPP14	85% 3-year PFS; 97% 3-year OS 84% 3-year PFS; 98% 3-year OS 68% 3-year PFS; 85% 3-year OS
GHSG HD18	3	1100	EscBEACOPP $\times 2 \rightarrow$ PET-2 PET-2 neg: escBEACOPP $\times 2$ escBEACOPP $\times 6$ PET-2 pos: escBEACOPP + rituximab escBEACOPP $\times 6$	93.0% 3-year PFS 91.4% 3-year PFS
LYSA AHL2011	3	810	EscBEACOPP $\times 6$ (arm 1) vs EscBEACOPP $\times 2 \rightarrow$ PET-2 (arm 2) PET-2 neg: ABVD $\times 4$ PET-2 pos: escBEACOPP $\times 4$	Interim results showed a 2-year PFS of 94% for 6 \times escBEACOPP, and 92% for 2 \times escBEACOPP followed by 4 \times ABVD

Abbreviations: ABVD, doxorubicin, bleomycin, vinblastine, dacarbazine; AVD, doxorubicin, vinblastine, dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone; BEACOPP-14, 14-day cycle; esc, escalated; PETpos, positive PET scan; PETneg, negative PET scan; RT, radiotherapy; PFS, progression-free survival; OS, overall survival; UK NCRI, United Kingdom National Cancer Research Institute; RATHL; response adapted therapy for Hodgkin lymphoma; GITIL, Gruppo Italiano Terapie Innovative nei Linfomi; GHSG, German Hodgkin Study Group; LYSA, Lymphoma Study Association.

BEACOPP (4+4). There was no difference in EFS or OS at 4 years. EFS was 63.7% for 8 cycles of ABVD vs 69.3% for escBEACOPP and baseline BEACOPP 4+4 ($p=0.312$); OS was 86.7% versus 90.3%, respectively ($p=0.208$).

Given the increased toxicity with escBEACOPP, two large trials have looked at limiting the number of cycles. GHSG HD 15 demonstrated that 6 cycles of BEACOPP escalated were superior to eight cycles in terms of OS and FFTF, with 5-year FFTF of 89.3% vs 85.4% for 6 and 8 cycles of escBEACOPP, respectively ($P=0.009$). PET-adapted de-escalation to reduce the number of cycles from 6–8 to 4 is also supported by the GHSG HD18 randomized, phase 3 trial. Among 2,101 patients treated with PET-directed reduction in therapy, 5-year PFS for 6–8 cycles vs 4 cycles resulted in similar PFS at 5 years; however, there were fewer severe infections and organ toxicities in patients treated with 4 cycles. De-escalation to ABVD is also supported by a small study of 45 patients with advanced-stage HL and an IPS score ≥ 3 . Patients with a CR or PR following 2 initial cycles of eBEACOPP were de-escalated to ABVD for 4 additional cycles. The 4-year PFS for early PET-negative and early PET-positive patients was 87% and 53%, respectively ($P=0.01$). See Table 22-11 for a summary of response-adapted trials in advanced-stage HL.

NCCN guidelines include consideration of escBEACOPP for patients younger than 60 years with advanced-stage HL and an IPS score ≥ 3 . It should be noted that this regimen is associated with mandatory G-CSF support, aggressive prophylactic antiemetics, dose-adaptation upon toxicity, and potential hospitalization during the first course for higher risk patients.

Brentuximab vedotin and chemotherapy combination

Brentuximab vedotin (BV) is an anti-CD30 antibody-drug conjugate. The initial phase 1 trial, combined with ABVD, resulted in significant pulmonary toxicity in 44% of patients, leading to removal of bleomycin from the regimen and treatment of an expanded cohort of patients with AVD plus BV. In a subsequent multicenter, randomized, phase 3 trial (ECHELON-1) of patients with stage III or IV cHL, patients were randomized to brentuximab vedotin, doxorubicin, vinblastine, and dacarbazine (A+AVD) ($n=664$) vs standard ABVD ($n=670$). Two-year modified PFS rates in the A+AVD and ABVD groups were 82.1% and 77.2%, respectively, resulting in a difference of 4.9 percentage points (HR for an event of progression, death, or modified progression, 0.77; $P=.03$). The A+AVD group had more neutropenia, but the rate of febrile neutropenia was lower among patients who received primary prophylaxis with granulocyte colony-stimulating factor vs those who did not (11% vs 21%). Peripheral neuropathy was more common

in A+AVD but was reversible in 67% of patients. Grade 3 or higher pulmonary toxicity was rare, being reported in <1% of patients receiving A+AVD and 3% of those treated with ABVD. Modified progression-free survival was a novel endpoint which included the use of modified progression events, defined as less-than-complete remission to front-line therapy (an end-of-treatment positron-emission tomography [PET] scan score of Deauville 3–5 *and* the delivery of subsequent treatment). This endpoint was criticized because a Deauville score of 3 is often considered to be a complete remission. Overall, six cycles of A+AVD is associated with a 5% lower combined risk of progression, death or to noncomplete response and use of subsequent anti-cancer therapy at 2 years compared to six cycles of ABVD. Limited follow-up showed no difference in OS.

A new modified BEACOPP variant incorporating brentuximab has been evaluated by the GHSG. The escBEACOPP variants met their coprimary efficacy endpoints in a phase 2 trial of advanced-stage cHL. In particular, the BrECADD regimen (brentuximab vedotin, etoposide, doxorubicin, cyclophosphamide, dacarbazine, and dexamethasone) was associated with a more favorable toxicity profile and was, therefore, selected to challenge the standard escBEACOPP regimen for the treatment of advanced cHL in the phase 3 HD21 study by the GHSG (NCT02661503). Longer-term follow-up will be needed from these randomized studies; however, these approaches offer an alternative strategy for improving therapy in patients with advanced-stage disease.

Radiation therapy or autologous transplantation as consolidation in stage III-IV HL

Several studies have examined the role of consolidative RT in patients with advanced-stage HL, and, to date, no study has demonstrated a clear OS advantage with combined modality therapy in patients responding to chemotherapy alone. The H89 Groupe d'Etude des Lymphomes de l'Adulte (GELA) study randomized patients achieving a response with MOPP/ABV hybrid or ABVPP either to two more cycles of chemotherapy or to STNRT. Ten-year OS was superior in the chemotherapy-alone arms, with 8% to 11% of patients achieving absolute improvement. Similarly, a randomized study of 739 patients with advanced-stage HL patients with a CR at the end of therapy, who were randomized to observation or IFRT, demonstrated no difference in 5-year OS ($P=.07$) or EFS ($P=.35$) in the RT group compared with the observation group. The GHSG HD12 trial randomized responding patients after BEACOPP with stage IIB-IV HL and with bulky or residual tumor on CT imaging either to additional consolidative RT or to no RT. Five-year FFTF was 87% in those patients who did not receive RT compared with 90% in the RT arm

($P=.08$). However, high-risk patients received RT irrespective of their group. Lastly, the GHSG HD15 trial evaluated PET-CTs in patients who had residual disease >2.5 cm after 6 to 8 cycles of BEACOPP. Those patients who were PET-positive received 30 Gy RT consolidation. In the 34% of patients with residual disease, 26% were PET-positive. PFS at 48 months was 93% in PET-negative patients and 86% in PET-positive patients. Although the irradiation of PET-positive patients with residual mass was not performed in a randomized fashion and there was no biopsy-proven active disease, the high tumor control rate suggests that radiating PET-positive disease after BEACOPP is a feasible approach that might also be applicable patients with residual PET positive masses at the end of therapy.

Several trials have also examined the role of frontline consolidative autologous transplantation to improve outcomes in patients with high-risk, advanced-stage HL. To date, none have demonstrated a clear role for transplantation.

Summary of frontline therapy for advanced-stage HL

For patients with advanced-stage disease, options for therapy include ABVD for 6 cycles or BEACOPP escalated for 4–6 cycles depending on interim PET response. Twelve weeks of Stanford V regimen is an alternative approach. Interim PET scans after 2 cycles of therapy are standard. Patients treated initially with ABVD, who attain a complete response on interim imaging, may be treated with as few as 2 cycles of ABVD followed by 4 cycles of AVD chemotherapy, omitting bleomycin. This strategy results in an equivalent PFS and OS compared to 6 cycles of ABVD based on the RATHL data. Patients with a positive PET scan after 2 cycles may be considered for escalation to 4 additional cycles of escalated BEACOPP with or without radiation for a PFS of approximately 60% to 67.5% at 3 years.

Initial treatment with escalated BEACOPP is an option in patients younger than age 60 and constitutes the standard of care in some countries. NCCN guidelines recommend consideration of initial treatment with escBEACOPP in patients younger than 60 years with an IPS score of ≥ 3 . The initial use of escalated BEACOPP in this setting results in improved disease control with absolute improvement in 5-year PFS over ABVD ranging from 13% to 18%; however, the survival benefit is less clear. BEACOPP is associated with increased toxicity in terms of infertility, grade 4 infections, and neutropenic fever. Patients with a complete response on PET scans after 2 cycles may be de-escalated to 4 cycles of ABVD with no compromise in FFS and OS, but reduction in treatment-related toxicity, including pulmonary and hematologic toxicities. Alternatively, the total number of treatment cycles of escBEACOPP may be reduced from 6 to 4.

Patients with bulky disease, a residual tumor >2.5 cm, following completion of therapy, and PET-positive disease at the end of therapy may be considered for consolidative radiation with 30 Gy based on the GHSG HD15 trial.

Future directions and upcoming studies in frontline therapy for HL

There have been 3 new targeted drugs approved for cHL in the past several years. Studies incorporating these agents into frontline therapy are ongoing including the novel combination of brentuximab and AVD (A+AVD) in advanced-stage cHL as described above. Longer follow-up for toxicity and survival data may help to clarify the role of this new regimen in the frontline treatment of cHL. Additional ongoing studies include the combination of PD-1 inhibitors with chemotherapy. Active trials include the combination of nivolumab and AVD in early-stage unfavorable cHL (NCT03004833), A(B) VD followed by nivolumab as frontline therapy for higher-risk patients (NCT03033914), and pembrolizumab followed by sequential AVD (NCT03226249) for all stages of cHL. Additionally, novel biomarkers of response have been identified. These include assessments of PD ligand expression on HRS cells, the tumor microenvironment, and peripheral blood, as well as soluble PD-L1, and alterations in chromosome 9p24.1.

KEY POINTS

- Therapeutic options for advanced-stage HL include ABVD for 6 cycles, escalated BEACOPP for 4–6 cycles, and Stanford V for 12 weeks followed by IFRT (36 Gy) to initially bulky site of disease.
- Escalated BEACOPP is associated with superior PFS compared with ABVD in patients with advanced-stage HL and may be considered as frontline therapy for patients younger than 60 years with high-risk disease; the benefit with respect to OS remains unclear.
- De-escalation of ABVD to AVD for PET2 negative patients is noninferior to ABVD alone. (RATHL study)
- Escalation from ABVD to BEACOPP for PET-2-positive patients results in superior outcomes compared with historic controls that continued ABVD therapy
- The ECHELON study using brentuximab vedotin and AVD (concurrent therapy) resulted in an absolute 5% lower combined risk of progression, death, noncomplete response, and use of subsequent anticancer therapy (ie, modified PFS) at 2 years compared with ABVD in patients with stage III/IV classical HL.
- Consolidative RT following chemotherapy is controversial in patients with advanced-stage HL treated with ABVD; in patients treated with BEACOPP, only PET-positive residual disease ≥ 2.5 cm should be irradiated.

Elderly HL

Elderly or older HL patients are defined as aged 60 years or older and constitute between 15% and 25% of all HL cases in population-based studies. Analyses studying different treatment regimens for newly diagnosed older HL patients over the past 15 years have reported 3-year progression-free survival (or failure-free survival [FFS]) rates of 50% to 67% with corresponding 3-year OS rates of 55% to 70%. Overall, there is no standard of care in this population. Valid therapeutic approaches include ABVD, AVD, CHOP, PVAG (prednisone, vinblastine, doxorubicin, and gemcitabine), and VEPEMB (vinblastine, cyclophosphamide, procarbazine, prednisone, etoposide, mitoxantrone, bleomycin).

The cause of poor outcomes for older HL patients is not completely understood, although poor outcomes have been attributed to a compilation of factors including presence of multiple comorbidities, poor performance status, disease/biologic differences (eg, more often mixed cellularity histology, EBV related, etc), inability to tolerate chemotherapy at full dose and schedule, and increased treatment-related toxicity and mortality. Compounding these challenges has been the underrepresentation of older patients in HL clinical trials over the prior several decades, which has been a barrier in the evaluation of disease biology and the discovery of more effective treatment strategies. Elderly patients have higher complication rates from chemotherapy and up to one-third may develop bleomycin lung toxicity (BLT) in comparison to <2% to 3% for younger patients. The risk of death from BLT is also higher, with up to 25% mortality.

In a GHSG analysis, elimination of bleomycin among elderly patients with early favorable HL resulted in decreased local control; however, OS rates exceeded 98%. Altogether, these data suggest that an upfront regimen of AVD may be considered, particularly in patients at high risk for BLT. Alternatively, bleomycin may be safely omitted after 2 cycles in those achieving an interim complete response without compromising efficacy as demonstrated in the phase 3 trial of PET-adapted therapy by the EORTC. Elderly patients with favorable-risk disease received either 2 cycles of ABVD or AVD each followed by IFRT compared with 4 cycles of ABVD. Grade 3/4 events and BLT were higher in patients receiving 4 cycles of therapy (65% overall); thus reduced therapy may be considered for the rare elderly patient with favorable disease.

Frontline trials using novel agents in elderly patients include a trial of brentuximab monotherapy, which demonstrated an objective overall response rate (ORR) of 92%; however, the risk of relapse was high. This study was amended to combine brentuximab vedotin with either

bendamustine or dacarbazine. Among 22 older HL patients treated with brentuximab vedotin and dacarbazine, there was a 2-year PFS of 50%. A recent multicenter phase 2 study in 48 elderly HL patients used initial single-agent brentuximab vedotin for 2 cycles, followed by standard AVD for 6 cycles with subsequent consolidative brentuximab vedotin for 4 cycles. The reported ORR and CR rates were 95% and 90%, respectively, and the 3-year PFS and OS rates were 84% and 93%, respectively. Furthermore, geriatric-based measures (eg, comorbidity score and loss of instrumental activities of daily living) were associated with patient outcome. These results are among the best reported in this patient population.

Pediatric HL

HL represents 7% of childhood cancers and is rare in children younger than 10 years but is the most common malignancy in the late teens. NS accounts for the majority of cases at approximately 70%. Mixed cellularity accounts for 30% and NLPHL accounts for 1% to 15%. LD is rare, except in association with HIV.

The vast majority of pediatric patients with HL are cured of their disease. Most children in the US with HL are treated in large referral centers, often in the context of clinical trials. As with adults, limiting exposure to radiation, avoiding alkylator-based regimens, such as MOPP, and reducing anthracycline exposure has been employed to reduce secondary malignancies, infertility, and other late toxicities given the long life expectancy of these patients. Patients are typically risk stratified with early-stage disease defined as stage I and IIA; advanced-stage disease includes patients with stage III and IV disease, bulky mediastinal disease, and all patients with B symptoms.

In the COG AHOD0031 study of 1,712 children with intermediate risk HL, patients were stratified according to rapid early response (RER) defined by at least 60% reduction in lymph node diameter by CT following 2 cycles of ABVE-PC (doxorubicin, bleomycin, vinblastine, etoposide, prednisone, cyclophosphamide). RERs received 2 additional cycles of ABVE-PC followed by PET/CT. Patients who were PET negative were randomized to observation vs IFRT. PET positive patients received IFRT. Slow early responders (SERs) who were PET-positive were treated with 2 additional cycles of ABVE-PC with or without 2 cycles of DECA (dexamethasone, etoposide, cisplatin and cytarabine). All SERs received IFRT. Overall, the 4 year EFS and OS were 85% and 98%, respectively; 87% and 99% for RERs and 77% and 95%, respectively, for SERs ($P < .001$). No difference in outcome was seen in RERs with or without the inclusion of IFRT,

with EFS at 4 years at 87% ($P=.87$). Slow early responders who were PET-positive benefitted from the addition of DECA with EFS 71% vs 55% ($P=.05$).

Other approaches in children include OEPA (vincristine, etoposide, prednisone and doxorubicin) for males and OPPA (vincristine, procarbazine, prednisone, and doxorubicin) for females, the Stanford V regimen, COPP/ABV, and escalated BEACOPP with or without low dose RT. Additional risk-adapted strategies to reduce the exposure to chemotherapy and RT are under investigation.

Therapy for relapsed or refractory HL

CLINICAL CASE

A 32-year-old man presented in 2009 with stage IVB cHL involving the bone marrow, liver, lungs, spleen, and multiple vertebrae. He received six cycles of ABVD with a negative PET or CT scan after cycles 2 and 4. A PET or CT scan 1 month after cycle 6 demonstrated a new liver lesion and biopsy confirmed HL. He received three cycles of ICE (ifosfamide, carboplatin, etoposide), achieved a second CR on PET or CT, and underwent autologous stem-cell transplantation in 2010. One year following transplantation, he developed progressive mediastinal and intra-abdominal adenopathy and new pulmonary nodules; biopsy of a retroperitoneal lymph node by endoscopic ultrasound confirmed recurrent HL. He then received brentuximab vedotin for 10 cycles and achieved a PR. Brentuximab vedotin initially was given every 3 weeks, but, due to neuropathy and neutropenia, the cycle length was increased to 4 weeks, and he remained on therapy for 16 cycles. He has one brother who is not an HLA match, but he does have several donor options through the National Marrow Donor Program registry.

Salvage therapy and autologous stem cell transplantation

More than 80% of patients with HL achieve complete remission with initial therapy; however, up to 40% of patients with advanced-stage disease and 10% to 15% with limited-stage disease may relapse and require additional treatment. Salvage chemotherapy followed by autologous stem cell transplantation (ASCT) remains the standard of care for patients with relapsed or refractory HL. High-dose chemotherapy and ASCT cure approximately 50% of patients, with long-term PFS in 60% of patients presenting with relapsed disease and 30% of those with primary refractory disease. There are no randomized data on optimal salvage regimens, and numerous options exist. Regimens include ICE, GVD (gemcitabine, vinorelbine, liposomal

doxorubicin), DHAP (dexamethasone, cytarabine, cisplatin), ESHAP (etoposide, methylprednisolone, cytarabine, cisplatin), GDP (gemcitabine, dexamethasone, and cisplatin), IGEV (ifosfamide, gemcitabine, vinorelbine, prednisolone), BeGEV (bendamustine, gemcitabine, etoposide, and vinblastine), mini-BEAM (carmustine, etoposide, cytarabine, melphalan), and Dexta-BEAM (dexamethasone, carmustine, etoposide, cytarabine, melphalan) (Table 22-12), with responses ranging from 70% to 90%.

There are two prospectively randomized trials comparing standard-dose with high-dose chemotherapy in patients with relapsed HL. The British National Lymphoma Group randomized 40 patients with relapsed disease either to BEAM followed by ASCT or to mini-BEAM alone; high-dose chemotherapy and ASCT demonstrated a significant PFS benefit ($P=0.005$). A larger trial of 161 chemosensitive patients randomized to two cycles of Dexta-BEAM and ASCT or two more cycles of Dexta-BEAM demonstrated a 3-year FFTF of 55% with transplantation compared with 34% without transplantation. Neither trial, however, demonstrated an OS benefit, perhaps because of limited follow-up or small patient numbers.

Chemosensitivity to second-line therapies further predicts worse survival with historical survivals reported to be as low as 3 months. OS was 39% at 5 years in patients with refractory disease to initial induction therapy compared to 67% in chemosensitive patients in one study. Additional features identifying patients at high risk for relapse post-transplantation include failure to attain PET negativity immediately prior to transplantation, short initial remission duration, and extra-nodal involvement.

A number of studies have demonstrated the prognostic value of PET/CT in this setting with EFS/PFS of 10% to 31% in patients who have PET positivity vs 68% to 93% for patients with a negative PET/CT immediately before SCT. It is reasonable to recommend 2–3 cycles of salvage chemotherapy, confirm response of disease by PET/CT, and then proceed with ASCT in responding patients. For those with progressive disease on PET/CT scans, alternative salvage regimens should be considered, and, if patients respond, autologous SCT is advocated.

A prospective phase 3 clinical trial, AETHERA, randomized a total of 322 cHL patients after treatment with high-dose chemotherapy and ASCT between consolidation treatment with brentuximab vedotin or placebo. Patients were included if they had at least one of the following risk factors for progression after ASCT: primary refractory HL, relapsed HL with initial remission duration <12 months, or extranodal involvement at the start of pre-transplantation salvage chemotherapy. Treatment was given at 1.8 mg/kg in 3-week intervals for up to 16 cycles. The

Table 22-12 Salvage combination chemotherapy regimens utilized for relapsed or refractory Hodgkin lymphoma

Regimen	Drugs	Method of administration	When administered	Cycle
GVD (not previously transplanted)	Gemcitabine 1,000 mg/m ²	IV	Days 1 and 8	Q 21 days
	Vinorelbine 20 mg/m ²	IV	Days 1 and 8	
	Liposomal doxorubicin 15 mg/m ²	IV	Days 1 and 8	
GVD (previously transplanted)	Gemcitabine 800 mg/m ²	IV	Days 1 and 8	Q 21 days
	Vinorelbine 15 mg/m ²	IV	Days 1 and 8	
	Liposomal doxorubicin 10 mg/m ²	IV	Days 1 and 8	
ICE	Ifosfamide 5,000 mg/m ²	IV over 24 h	Day 2	Q 14–21 days
	Mesna 5,000 mg/m ²	IV over 24 h	Day 2	
	Etoposide 100 mg/m ²	IV	Days 1–3	
	Carboplatin AUC=5 (maximum dose of 800 mg)	IV	Day 2	
DHAP	Dexamethasone 40 mg	IV/PO	Days 1–4	Q 21 days
	Cisplatin 100 mg/m ²	IV over 24 h	Day 1	
	Cytarabine 2,000 mg/m ²	IV every 12 h	Day 2	
ESHAP	Etoposide 40 mg/m ²	IV	Days 1–4	Q 21 days
	Methylprednisolone 500 mg	IV	Days 1–5	
	Cytarabine 2,000 mg/m ²	IV	Day 5	
	Cisplatin 25 mg/m ²	CIV	Days 1–4	
Mini-BEAM	BCNU (carmustine) 60 mg/m ²	IV	Day 1	Q 21–28 days
	Etoposide 75 mg/m ²	IV	Days 2–5	
	Cytarabine 100 mg/m ²	IV every 12 h	Days 2–5	
	Melphalan 30 mg/m ² (maximum of 50 mg)	IV	Day 5	
Dexa-BEAM	Dexamethasone 24 mg	PO	Days 1–10	Q 28 days
	BCNU (carmustine) 60 mg/m ²	IV	Day 2	
	Melphalan 20 mg/m ²	IV	Day 3	
	Etoposide 200 mg/m ²	IV every 12 h	Days 4–7	
	Cytarabine 100 mg/m ²	IV every 12 h	Days 4–7	
	G-CSF 300–480 mg	SQ	Day 9 until WBC > 2,500/μL	
IGEV	Ifosfamide 2,000 mg/m ²	IV	Days 1–4	Q 21 days
	Gemcitabine 800 mg/m ²	IV	Days 1 and 4	
	Vinorelbine 20 mg/m ²	IV	Day 1	
	Prednisolone 100 mg	PO	Days 1–4	
GDP	Gemcitabine 1,000 mg/m ²	IV	Days 1 and 8	Q 21 days
	Cisplatin 75 mg/m ²	IV	Days 1 and 8	
	Dexamethasone 40 mg	PO	Days 1–4	
ChlVPP	Chlorambucil 6 mg/m ²	PO	Days 1–14	Q 28 days
	Vinblastine 6 mg/m ²	IV	Days 1 and 8	
	Procarbazine 100 mg/m ²	PO	Days 1–14	
	Prednisone 40 mg	PO	Days 1–14	

Table 22-12 (continued)

Regimen	Drugs	Method of administration	When administered	Cycle
BeGEV	Bendamustine 90 mg/ m ²	IV	Days 2,3	Q 21 days
	Gemcitabine 800 mg/m ²	IV	Days 1, 4	
	Vinorelbine 20 mg/m ²	IV	Day 1	
	Prednisolone 100 mg	PO	Day 1–4	
Brentuximab vedotin	1.8 mg/kg (capped at maximum of 100 kg)	IV	Day 1	Q 21 days

Source for GVD: Bartlett NL et al. *Ann Oncol*. 2007;18:1071–1079. Source for ICE: Moskowitz CH et al. *Blood*. 2001;97:616–623. Source for DHAP: Josting A et al. *Ann Oncol*. 2002;13:1628–1635. Source for Mini-BEAM: Kuruvilla J et al. *Cancer*. 2006;106:353–360. Source for Dexa-BEAM: Josting A et al. *Ann Oncol*. 1998;9:289–295. Source for IGEV: Santoro A et al. *Haematologica*. 2007;92:35–41. Source for GDP: Kuruvilla J et al. *Cancer*. 2006;106:353–360. Source for Ch1VPP: Vose JM et al. *J Clin Oncol*. 1991;9:1421–1425. Source for brentuximab vedotin: Chen R et al. *J Clin Oncol*. 2011;29:8031 [abstract]. Source for BeGEV: Santoro A et al. *J Clin Oncol*. 2016; 34:3293–3299.

AUC, area under the concentration-time curve; CIV, continuous intravenous; IV, intravenous; PO, per os (by mouth); Q, every; SQ, subcutaneous.

hazard ratio of this trial was 0.57 ($P=.001$) with a median PFS in the brentuximab vedotin arm of 42.9 months and 24.1 months in the group treated with placebo. Although there was more toxicity with brentuximab vedotin, the study demonstrated feasibility with a median of 15 cycles of brentuximab vedotin received. Thus, consolidation treatment with brentuximab vedotin has been established as a treatment option for patients with higher risk of relapse following ASCT.

Therapeutic options for patients relapsing after autologous stem cell transplantation or not eligible for transplantation

Historically, patients who relapsed following ASCT had poor outcomes, with a median survival of 1–2 years. However, several novel agents have recently been approved by the FDA for patients relapsing after ASCT or not eligible for ASCT. These therapeutic agents include the CD30 antibody-drug conjugate brentuximab vedotin as well as the PD-1 inhibitors pembrolizumab and nivolumab. These agents have largely replaced salvage chemotherapy as the preferred treatment in these settings due to their efficacy and tolerability. A recent retrospective analysis demonstrated that treatment with these novel targeted agents was associated with significant improvement in OS (median survival of 85.6 months vs 17.1 months, $P=.015$). Other factors that increased likelihood of survival at relapse included post-ASCT radiation therapy (34.1 vs 17.0 months; $P=.015$).

Brentuximab vedotin

The FDA approved brentuximab vedotin (BV) in 2011, a novel anti-CD30 drug-antibody conjugate for the treatment of patients with relapsed or refractory HL after previous ASCT. BV is composed of a CD30 antibody conju-

gated by a plasma-stable link to the antimicrotubule agent, monomethyl auristatin E (MMAE). In a pivotal phase 2 study with 102 relapsed (29%) or refractory (71%) cHL patients who had received a median of 3.5 prior therapies (range 1–13), the overall response rate (ORR) was 75%, with a 33% achieving CR. OS was 40.5 months and grade 3–4 toxicities consisted of sensory neuropathy (8%), neutropenia (20%), and thrombocytopenia (8%). BV may be administered for up to 16 cycles, with dose reductions or delays, if needed, for myelosuppression or neuropathy.

Anti-PD-1 therapy

Nivolumab is a high affinity, fully human, IgG4 (S228P) monoclonal antibody directed against PD-1. As with pembrolizumab, nivolumab's activity against PD-1 allows dual blockade of its major ligands, PD-L1 and PD-L2. It is approved for patients with cHL who have relapsed or progressed after autologous SCT and posttransplantation brentuximab vedotin treatment on the basis of phase 1 and 2 trials. The phase 2 clinical trial, CheckMate 205, assessed patient who had failed prior ASCT and had either relapsed or failed brentuximab vedotin. Overall, 66% of patients achieved a response. Therapy was well tolerated with 51 (64%) of patients remaining on treatment at last follow up. The recommended dose schedule is 240 mg every 2 weeks. Additionally, 44 patients on CheckMate205 subsequently proceeded to allogeneic SCT. The 6-month cumulative incidence of treatment related mortality (TRM) was 13%, and 7% had disease progression. The cumulative 6-month incidence of grade 3–4 acute graft-versus-host disease (GVHD) was 20%, and 15% had chronic GVHD. Univariate analysis did not identify associations between time from last dose of nivolumab to allogeneic SCT and TRM. These data appeared grossly comparable with historical relapsed/refractory HL cohorts receiving allografts without

preceding PD-1 blockade. The 6-month PFS and OS estimates were 82% and 87%, respectively. However PD-1 therapy after allogeneic transplantation may be associated with higher rates of severe GVHD (see “Allogeneic transplant” below).

Pembrolizumab is also approved for the treatment of refractory adult and pediatric cHL that has relapsed after at least 3 lines of therapy. Pembrolizumab is a highly selective humanized IgG4-kappa isotype antibody that is also directed against PD-1, and it demonstrated impressive response rates in phase 1 and 2 clinical trials of relapsed cHL. The phase 2 study KEYNOTE-087 utilized a flat dose of 200mg every 3 weeks. Pembrolizumab was studied in 3 cohorts: (1) following relapsed after ASCT and subsequent brentuximab vedotin (BV); (2) after failure of salvage chemotherapy and BV, and ineligible for ASCT; and (3) in brentuximab-naïve patients following ASCT. A total of 210 patients were enrolled and received a median of 13 treatment cycles. Responses per central review were as follows: ORR of 69.0 and CR rate of 22.4%. By cohort, ORRs were 73.9% for cohort 1, 64.2% for cohort 2, and 70.0% for cohort 3. There were some with durable response, with 31 patients maintaining a response of ≥6 months.

Radiation

Radiotherapy should also be considered in the setting of relapsed HL in highly selected patients with limited-stage disease at relapse who may not be eligible for ASCT due to age and comorbid conditions. In a retrospective analysis of salvage RT used in 100 patients at first treatment failure, 5-year FFTF and OS were 28% and 51%, respectively, with RT alone. For younger patients with relapsed HL, because of potential risks of second malignancies within the radiation field and improved survival with ASCT, RT alone is not recommended at first relapse. IFRT, however, should be considered in these patients as consolidation post-ASCT to bulky, nonirradiated sites or to sites of relapsed limited stage disease in previously nonirradiated fields.

Chemotherapy

A number of single-agent regimens are used in the palliative setting and include vinblastine, etoposide, gemcitabine, and vinorelbine. With vinblastine, 4–6 mg/m² weekly or every 2 weeks until disease progression or toxicity, response rates as high as 59% and median EFS of 14 months have been reported. Gemcitabine and vinorelbine both have single-agent activity in 39% to 50% of patients. The histone deacetylase inhibitor panobinostat also has activity in this population, including multiply relapsed disease; however, HDAC inhibitors are not FDA-approved in HL. Selected patients with nonbulky lymphadenopathy and no organ in-

volvement who are otherwise asymptomatic also could be observed in this setting.

Allogeneic transplantation

Allogeneic transplantation has been used for patients with relapsed HL after prior ASCT although the presence of a graft-versus-HL effect remains controversial. Most trials of allogeneic SCT in HL demonstrate 2-year PFS rates of 30% and OS of 35% to 60%. Overall, for selected patients with available donors who are at least a good PR, reduced-intensity allogeneic SCT is an option after prior ASCT and may lead to prolonged DFS in 18% to 32% of patients.

The widespread use of immunotherapy and, in particular, PD-1 inhibitors in the peritransplantation period have demonstrated notable interaction on immunologic response, recovery, and post-transplantation treatment outcomes. A retrospective analysis from the University of Colorado assessed 29 cHL patients receiving anti-PD-1 therapy for relapse post-allogeneic SCT. The ORR to therapy was 77%, but there was 26% deaths due to new onset GVHD, including 55% treatment emergent GVHD (6 acute, 4 overlap, 7 chronic). Nine patients with grade 3/4 toxicities had a poor response to systemic GVHD treatments. Correlative analysis further demonstrated persistent immunologic changes consistent with immune alteration from PD-1 therapy. Caution should be exercised when using PD-1 inhibitors at relapse after allogeneic SCT for possible flare of GVHD and other immune-related toxicities.

Other novel therapies

Several additional novel treatments are being investigated for patients who are ineligible for or who have relapsed following transplantation. Interim results of the phase 1/2 study combining brentuximab vedotin and nivolumab. Sixty-two patients with HL in first relapse were treated with up to 4 cycles of combination therapy, and 61% achieved a CR, with an ORR of 82%. The combination was well tolerated with fewer than 10% of patients requiring treatment with systemic steroids for immune related adverse events. Several other combinations with PD-1 therapies are ongoing, including pembrolizumab with radiation therapy (NCT0317991), pembrolizumab and lenalidomide (NCT02875067), pembrolizumab and ibrutinib (NCT02950220), as well as a head-to-head comparison of pembrolizumab vs brentuximab vedotin for patients in first relapse (KEYNOTE-204). Given the immunologic properties of HL and high response rates of PD-1 inhibitors, additional immunotherapies are currently being evaluated, which include lenalidomide and ipilimumab alone or in combination with other agents. Lenalidomide is an immunomodulatory agent that has demonstrated

activity in several hematologic malignancies, including HL. The largest trial of single-agent lenalidomide ($n=38$) demonstrated an ORR of 19% and CR rate of 3%. Lenalidomide combined with bendamustine (Leben combination) resulted in an ORR of 75% and a CR rate of 44%.

KEY POINTS

- Fit elderly/older HL patients should be considered for sequential brentuximab vedotin therapy given before and after standard AVD chemotherapy; less-fit older patients not amenable to standard combination chemotherapy may be considered for treatment with brentuximab vedotin with dacarbazine.
- Salvage chemotherapy followed by autologous transplantation offers superior PFS compared with chemotherapy alone in patients with relapsed, chemosensitive HL.
- Post-transplantation brentuximab vedotin is recommended for patients with a high risk of post-transplantation relapse based on the phase 3 AETHERA trial.
- Brentuximab vedotin leads to overall response rates of 75% in patients with relapsed HL following autologous transplantation.
- Nivolumab and pembrolizumab are anti-PD-1 antibodies approved for patients with relapsed/refractory Hodgkin lymphoma.
- Caution should be used when considering PD-1 inhibitors in the peritransplantation setting due to concerns of increased risk and severity of GVHD, especially post-SCT.

Nodular lymphocyte-predominant HL

CLINICAL CASE

A 19-year-old college lacrosse player presented with left-sided cervical adenopathy and a large parotid mass of 6 cm, initially thought to be secondary to acute infectious mononucleosis. The mass failed to improve despite 6 months of intermittent steroids and antibiotics, and subsequent biopsy demonstrated atypical large cells with large nuclei that were CD20-, PAX-5-, BCL-2-, and CD45-positive and CD15- and CD30-negative, consistent with NLPHL. CTs of the C/A/P demonstrated bilateral cervical adenopathy but no other sites of disease; bone-marrow biopsy was negative.

NLPHL is an uncommon subtype of HL, representing about 5% of cases, with unique pathologic features distinguishing it from cHL. Because of the rare occurrence of this malignancy, presentation, treatment, and patient out-

comes are not well described. In a retrospective analysis of 8,298 patients enrolled on clinical trials for HL through the GHSG, 394 patients had NLPHL. In this series, the median age at diagnosis was 37 years, 75% of patients were male, and 79% had early-stage disease. The presence of B symptoms or bulky disease is unusual and is observed in <10% of patients. Unlike cHL, patients with NLPHL typically have peripheral adenopathy (axillary or inguinal) at diagnosis rather than central or mediastinal involvement; nodal involvement is not contiguous, and extranodal involvement is uncommon.

An association exists with this subtype of lymphoma and a benign condition, progressive transformation of germinal centers, as well as with NHL, particularly T-cell-rich B-cell lymphoma and diffuse large B-cell lymphoma. Progressive transformation of germinal centers is described as lymph nodes with large, well-defined nodules with an excess of B-cells or germinal centers overrun by lymphocytes. Progressive transformation of germinal centers may be observed before, simultaneously with, or following a diagnosis of NLPHL. This entity is thought to be a benign condition, but, because it occurs concurrently or following a diagnosis of NLPHL, biopsy of recurrent adenopathy always is required with this disease to confirm relapse.

Likewise, T-cell-rich B-cell lymphoma can occur simultaneously or in succession and may be confused with NLPHL. Because ~5% to 10% of patients with NLPHL eventually develop NHL, biopsy of recurrent lymph nodes is necessary to determine optimal therapy at relapse. Overall survival is similar; however, there are more frequent relapses in NLPHL. Additionally, late relapses >1 year after therapy are observed more commonly in patients with NLPHL (7.4%) compared with patients with cHL (4.7%).

No standard frontline or relapsed therapy exists for NLPHL, although a number of options are available with excellent outcomes. Adverse prognostic factors in NLPHL include advanced-stage disease, hemoglobin <10.5 g/dL, age >45 years, and lymphopenia (<8% of total white cell count). Additionally, one study showed that splenic involvement was associated with an inferior 10-year TTP in NLPHL (48% vs 71%; $P=.049$) as well as an increased cumulative incidence of secondary aggressive lymphoma ($P=.014$).

For early-stage NLPHL, IFRT alone is recommended, especially for patients with peripherally located stage IA disease. Two small retrospective studies of limited-stage NLPHL, with a total of 245 patients, found no benefit of combined modality therapy over radiation alone. In contrast, one retrospective comparison of 32 patients treated with

RT alone versus 56 patients receiving CMT with ABVD for 2 cycles and RT demonstrated improved PFS survival (65% vs 91%, $P=.0024$) with CMT. Therefore, most series support favorable outcomes with RT alone in early stage IA NLPHL. Because of the risks of second malignancies and the excellent long-term outcomes observed in patients with LPHL, in selected patients in whom the disease is completely resected, observation could also be discussed as alternative to IFRT.

Chemotherapy alone may be used for non-stage IA patients or for those with very high risk of late complications of RT due to the field size of RT required. Cyclophosphamide, vinblastine, and prednisolone (CVP) or single agent rituximab may also be considered with response rates of 100% but a slightly shorter PFS compared to radiation. However, these early-stage patients who relapse after chemotherapy can be effectively salvaged with additional chemotherapy and RT, and such an approach may reduce the rates of second malignancies.

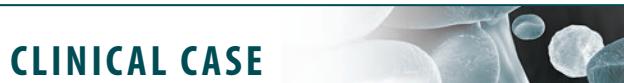
In the advanced-stage setting, chemotherapy options include six cycles of ABVD or BEACOPP, or alkylator regimens (CVP or CHOP). Rituximab may be given alone as a single agent or in combination with chemotherapy. All these strategies result in response rates nearing 100%. Advani, et al, reported data using single-agent rituximab induction weekly for 4 weeks followed by maintenance rituximab once every 6 months for 2 years for previously treated or newly diagnosed NLPHL. At median follow-up of 9.8 years, the median OS was not reached. Of patients who experienced relapse, 39% of NLPHLs had transformed to an aggressive B-cell lymphoma. R-CHOP was associated with estimated 5- and 10-year PFS rates of 88.5% and 59.3%, respectively, in NLPHL. With a median follow-up of 6.7 years, no patient treated with R-CHOP experienced transformation. These regimens frequently are utilized as frontline or salvage therapy for stage III-IV NLPHL.

KEY POINTS

- NLPHL is associated with progressive transformation of germinal centers (a benign condition) and also transformation to diffuse large B-cell or T-cell-rich B-cell NHL; therefore, biopsy at relapse is necessary.
- Unlike HL, NLPHL is associated with noncontiguous nodal spread and late relapses.
- No standard therapy exists for NLPHL; IFRT is used for stage IA disease; CMT or observation for other early-stage disease; combination chemotherapy with rituximab (including R-CHOP) for advanced-stage disease; and single-agent rituximab in the relapsed setting.

Follow-up of patients with HL

CLINICAL CASE



An 18-year-old nonsmoking man with no history of cardiac disease, diabetes, or elevated cholesterol presented with bulky stage IIB CHL involving the mediastinum and bilateral supraclavicular nodes. He received six cycles of ABVD, followed by mantle-field irradiation. He was followed every 6 months with CT scans for 2 years and then annually with CT scans until year 5 with no recurrence. After his fifth year, he relocated for a new job opportunity and was followed only as needed by a primary care physician (PCP). Approximately 15 years after diagnosis, at age 33, he acutely developed nausea and chest discomfort and was seen in a local emergency room. Because of lack of cardiac risk factors and initially normal electrocardiogram and troponin, he was admitted to a nonteaching service for observation with the thought that this was gastrointestinal discomfort. Subsequent troponin levels continued to rise, and the patient was urgently taken to cardiac catheterization where he was found to have a 90% occluded left-anterior descending artery.

Secondary, late therapy-related effects in HL survivors include hypothyroidism, fertility issues, secondary cancers, and cardiovascular disease. The risks of second malignancies and cardiovascular disease continue 40 years after diagnosis. Therefore, monitoring of late complications is a lifelong endeavor for HL survivors. Follow-up of patients with HL must address both the risk of relapse as well as potential late complications of therapy. In a study of 1,261 patients treated for HL before the age of 41 from 1965–1987, 534 patients died, causes of death being HL (54%), second malignancies (22%), and cardiovascular disease (9%). The likelihood of HL recurrence declines sharply after 3 years, whereas the incidence of second malignancies and cardiovascular disease continually increased beginning 10–15 years from the start of treatment and continuing beyond 40 years after treatment. Within the first 5 years after diagnosis, patients should be monitored for HL recurrence with history and symptom-directed evaluation, physical examinations, and laboratory testing (CBC, platelets, chemistries, and ESR if elevated at initial diagnosis) every 2–3 months for the first 2 years and every 3–6 months during years 3–5.

Several studies have demonstrated no survival benefit with routine CT surveillance in patients achieving a CR at the end of therapy. Follow-up PET/CTs demonstrate a high false-positive rate, with an overall positive predictive value (PPV) of only 28%, limiting its utility as a follow-up tool for HL. Therefore, with the low risk for relapse in HL

and no demonstrated survival benefit with routine surveillance imaging, follow-up should consist of history and physical examination with only symptom-directed imaging during the first 5 years after HL diagnosis. At most, CT scanning every 6 months for a maximum of 2 years after original diagnosis may be considered for surveillance.

In a meta-analysis, second cancers were more commonly encountered in patients receiving radiation-containing treatment compared with chemotherapy alone, with no significant decreases in the second malignancy rate observed with more modern radiation techniques so far (Figure 22-5). Therefore, any patient receiving previous RT should be monitored for a second malignancy and cardiovascular disease (Table 22-13). The risk of secondary breast cancers is associated with young age at the time of radiation, and women younger than 30 years are particularly at risk. Lung cancer risk is increased in patients receiving mediastinal radiation, particularly if they have a smoking history, and chest imaging annually should be considered for these patients at greatest risk. Cardiovascular disease, including increased risk of coronary artery disease and valvular disease, also is observed in HL survivors, particularly after mediastinal radiation or anthracycline-based chemotherapy, starting about 5 years after treatment (Figure 22-6). Although optimal screening strategies are unclear, monitoring and aggressive management of cardiovascular risk factors, including smoking, hypertension, diabetes, and hyperlipidemia, is recommended with consideration of a baseline stress test or echocardiogram (Table 22-13).

Other late toxicities associated with RT include hypothyroidism, which can occur in up to 50% of patients, and radiation pneumonitis or lung fibrosis (3%–10% of patients). Annual thyroid function tests are recommended for patients with radiation to the neck or upper mediastinum, and evaluation for pulmonary fibrosis should be considered in symptomatic patients.

Secondary MDS and leukemia affect up to 1% of patients receiving ABVD and have been observed in up

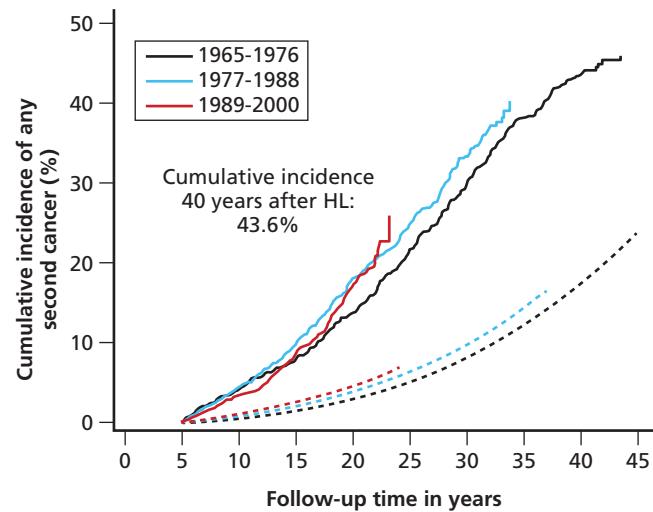


Figure 22-5 Cumulative incidence of solid malignancy after HL according to calendar period of treatment. Redrawn from van Leeuwen FE, Ng AK, *Hematology Am Soc Hematol Educ Program*. 2016;2016:323–330, with permission from the publisher.

Table 22-13 NCCN recommendations for monitoring and screening beyond 5 years*

Category	Recommendation
General health maintenance	Annual history and physical BP and laboratory studies (CBC with differential, chemistry panel, fasting glucose, TSH if radiation near neck, and biannual lipids)
Vaccinations	Annual influenza and pneumococcal, <i>Haemophilus influenzae</i> type b conjugate after 5–7 years if treated with splenic RT or splenectomy and/or 6 months following stem cell transplantation (including hepatitis B virus, diphtheria, acellular pertussis, and tetanus; measles, mumps, rubella, and varicella live vaccines may be given for seronegative patients 2 years after transplant, if no immunosuppressive therapy for at least 6 months)
Cardiovascular	Consider cardiac stress test/echo at 10-year intervals after treatment
Carotid	Consider carotid ultrasound if neck radiation
Breast cancer	Initiate 8–10 years posttherapy, or age 40 years, whichever comes first, with MRI in addition to mammography for women who received irradiation to the chest and/or axilla between ages 10 and 30 years
Cervical, colorectal, endometrial, lung, prostate cancer	Per standard ACS cancer screening guidelines
Miscellaneous	Counseling for reproduction, health habits, psychosocial, and skin cancer risk

*Full treatment summary should be completed for each patient with consideration for referral to a survivorship clinic.

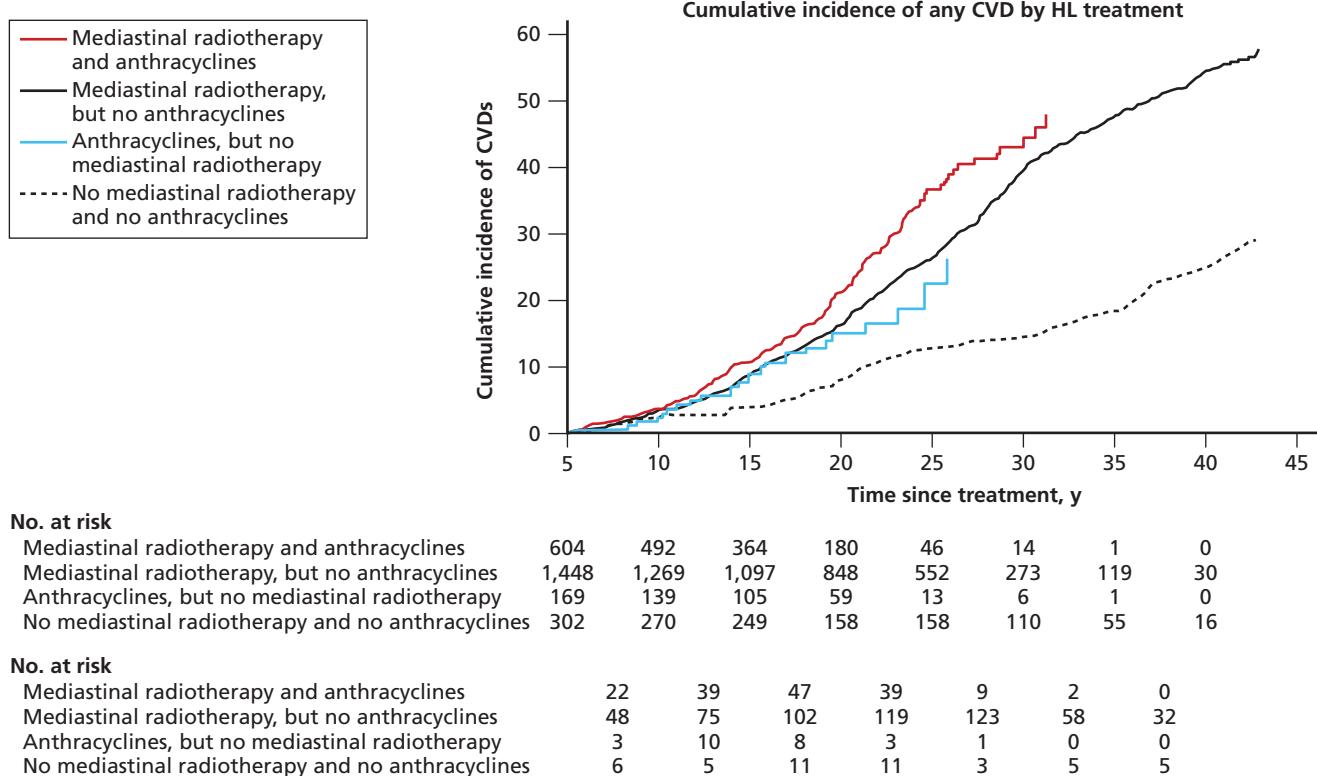


Figure 22-6 Cumulative incidence of CVDs after HL according to treatment, with death from any cause as a competing risk. Redrawn from van Leeuwen FE, Ng AK, *Hematology Am Soc Hematol Educ Program*. 2016;2016:323–330, with permission from the publisher.

to 3% of patients treated with eight cycles of BEACOPP escalated. In contrast, with 6 cycles of BEACOPP escalated and radiation only to PET-positive residual disease ≥ 2.5 cm, the AML/MDS rate was only 0.2%. With respect to fertility, patients treated with BEACOPP have a high risk of infertility depending on the age at treatment and the number of cycles received. All patients receiving chemotherapy should be counseled about this risk and referred for sperm banking or reproductive endocrinology evaluation. ABVD does not appear to affect female fertility significantly. Several large studies by the GHSG demonstrated preserved gonadal function, return of menses following chemotherapy, and equal numbers of pregnancies in female patients treated with ABVD compared to population-based controls.

Anthracycline-related cardiotoxicity in the absence of mediastinal RT is rare in this patient population because the total cumulative dose of doxorubicin administered is 300 mg/m² or less. An evaluation of left-ventricular function is typically obtained before the initiation of chemotherapy, although asymptomatic cardiac dysfunction is uncommon in this patient population, especially for younger

patients. Additionally, there is an increased risk of myocardial infarction (MI) for 25 years after treatment with anthracyclines (SMR for MI of 7.8 with ABVD alone; and 12.1 for ABVD and RT). Aggressive management of other cardiac risk factors is recommended.

In addition to these risks, patients who undergo ASCT for relapsed disease should be monitored for risks of secondary leukemia, other secondary malignancies, hypogonadism and its complications, including declines in bone mineral density; these patients also should be considered for revaccination. In addition, patients typically experience hypogonadism post-transplantation, and monitoring for consequences of hormonal deficiency is recommended, including monitoring for bone mineral density reduction using DEXA scanning.

Immunity typically wanes post-autologous transplantation, and it is recommended that patients receive pneumococcal, tetanus, *Haemophilus influenzae* type b, hepatitis B, and annual influenza vaccinations. Measles, mumps, and rubella (MMR) and varicella vaccinations can be considered in immunocompetent patients no sooner than 24 months posttransplantation (Table 22-13).

KEY POINTS

- ABVD does not significantly impact fertility, while escalated BEACOPP is expected to reduce fertility in direct proportion to the number of cycles received.
- Treatment summaries should be completed for each patient and consideration given to referral to a survivorship clinic.
- Routine follow-up for HL survivors consists of history and directed physical examination with symptom-directed laboratory testing or imaging. Surveillance imaging and laboratory testing have not been shown to improve survival or to increase detection of relapsed disease.
- Monitoring for secondary malignancies and cardiovascular disease is a lifelong endeavor for HL survivors. Annual mammography is recommended starting 8–10 years after completion of treatment in women treated with chest or axillary radiation. Smoking cessation, cardiovascular risk assessment, and monitoring for hypothyroidism are recommended, particularly in patients receiving mediastinal or neck radiation. Referral to specialty survivorship clinics should be considered for HL survivors.

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23

Non-Hodgkin lymphomas

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Overview of lymphocyte development and classification of lymphoid malignancies

The lymphoid system forms the backbone of the human immune system, contributing to both the innate (nonspecific) immune response through natural killer (NK) cells and the adaptive (specific) immune response through B and T cells. Non-Hodgkin lymphomas are malignancies that arise from these cells, generally grouped as B-cell lymphomas and T-cell lymphomas. Knowledge of B- and T-cell development is important in understanding the biology and, in turn, in providing insight into the behavior of the numerous subtypes of these lymphomas that are derived from their normal B- and T-cell counterparts.

B-cell development and the biology of B-cell lymphomas

Common lymphoid progenitors in the bone marrow derived from hematopoietic stem cells are the source of B- and T-cells. Unlike T-cells, full B-cell maturation occurs in the bone marrow and begins with recombination of the *V*, *D*, and *J* gene segments of the immunoglobulin heavy chain (IgH) followed by the light-chain genes in order to generate a functional immunoglobulin that is expressed on the cell surface as B-cell receptor (BCR). The survival and maturation of B cells in the bone marrow, as well as the differentiation of mature B cells that have exited the bone marrow, are dependent on operative BCR signaling. Importantly, BCR signaling has also been found to be necessary for lymphoma development and evolution with many mature B-cell malignancies showing sensitivity to kinase inhibitors which disrupt BCR signaling.

Collectively, the primary function of B cells is to generate a vast diversity of immunoglobulins. Generating this diversity begins with the combinatorial diversity produced from random *V*, *D*, and *J* rearrangements. Combinatorial diversity is amplified by junctional diversity produced by the action of terminal deoxynucleotidyl transferase (TdT) where nucleotides are randomly added or deleted at the sites of *V*, *D*, and *J* fusion. Successful rearrangement of the heavy and light immunoglobulin chains (kappa or lambda) results in expression of functional IgM and IgD on the surface of mature B cells that exit the marrow. These mature, but antigen-naïve, B cells then gain additional diversity when exposed to antigens in

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Off-label drug use: Lenalidomide in follicular lymphoma; rituximab in hairy cell leukemia; bendamustine, brentuximab vedotin, gemcitabine, ibrutinib, lenalidomide and oxaliplatin in DLBCL; ibrutinib, lenalidomide, temozolamide and thiopeta in PCNSL; pembrolizumab in PMBCL; alemtuzumab, gemcitabine, lenalidomide and liposomal doxorubicin in PTCL; mogamulizumab in ATLL; crizotinib in ALK+ALCL.

the germinal centers of secondary lymphoid organs, such as lymph nodes, mucosa-associated lymphoid tissue, or the spleen. Here, somatic hypermutation occurs in the V genes of the heavy and light chains, fine-tuning their affinity to their cognate antigens. B cells expressing immunoglobulin with just the right amount of antigen affinity, differentiate to memory B cells and plasma cells while all the others undergo apoptosis. Finally, class switching occurs in the germinal center and involves changing the heavy chain that is expressed to produce IgG, IgA, or IgE.

The classification of B-cell lymphomas is based, in part, on the resemblance of a given lymphoma subtype to a particular stage in B-cell development and differentiation which reflects their origin and informs their biology (Figure 23-1). Distinct stages of B-cell development and differentiation are characterized by cytologic features, ex-

pression patterns of differentiation markers, and the B-cell antigen receptor (BCR). These characteristics form the basis of pathologic diagnosis of lymphoid neoplasms. For example, B-lymphoblastic leukemia/lymphoma arises from an immature B cell (Figure 23-1) and, accordingly, diagnosis requires the identification of immature B cells that have morphologic characteristics of blasts; coexpress B-cell markers, such as CD19, with markers of immaturity, such as TdT and CD10; and do not express BCRs on their surface. Likewise, follicular lymphoma (FL) arises from a germinal-center B cell (Figure 23-1) and has morphologic characteristics of nodular growth, resembling B-cell follicles, while expressing the germinal-center marker CD10 with surface IgM, IgD, IgG, or IgA.

The transformation of normal B cells into their malignant counterparts is closely linked to the essential role of B

Figure 23-1 Schematic representation of B-cell differentiation (WHO 2017). CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; GC, germinal center; MALT, mucosa-associated lymphoid tissue. Reproduced with permission from Harald Stein.

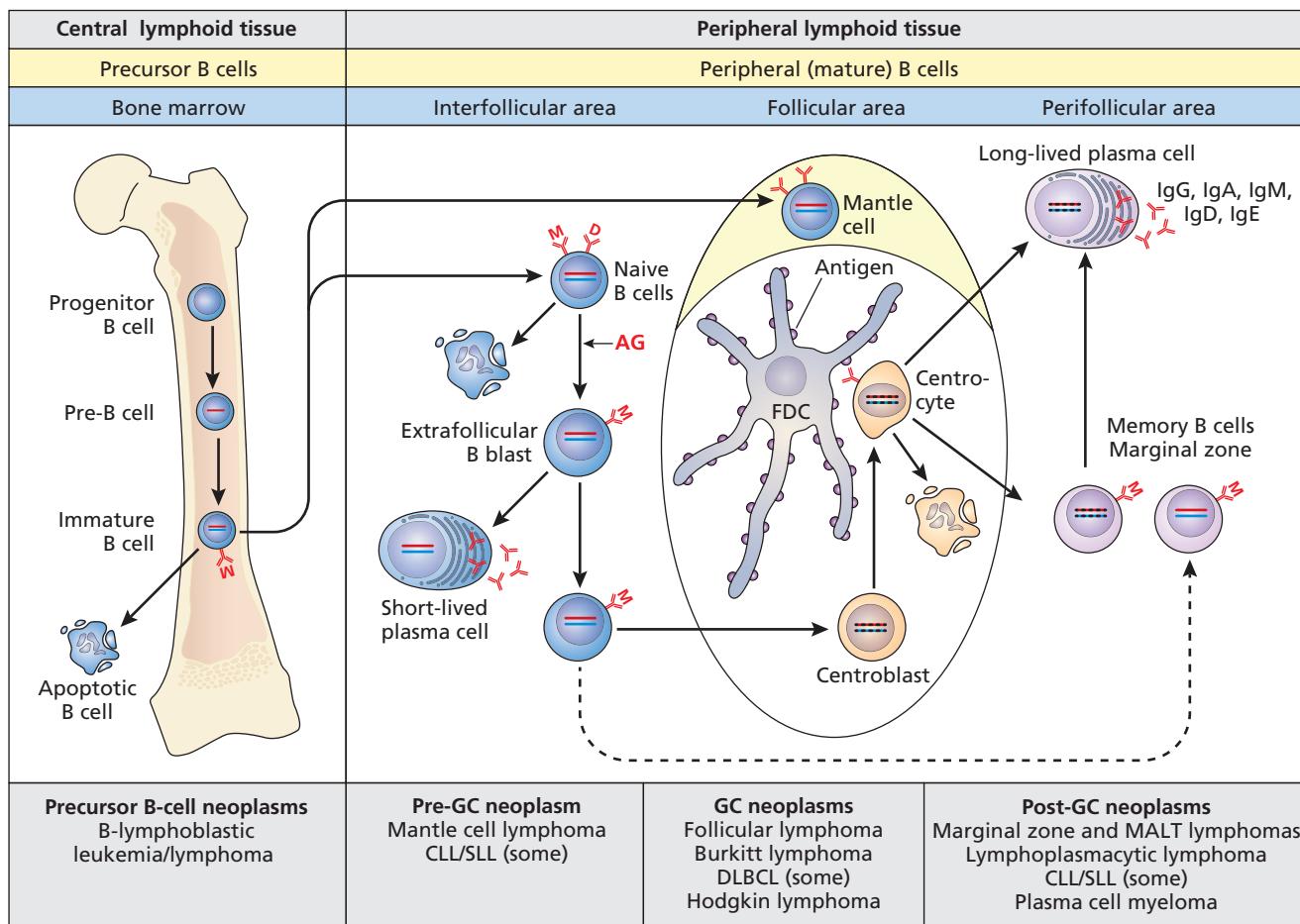


Table 23-1 Risk factors in the development of non-Hodgkin lymphoma

Viral infection	EBV, HTLV-1, HHV-8, hepatitis C virus
Bacterial infection	<i>Helicobacter pylori</i>
	<i>Chlamydophila psittaci</i>
Impaired/altered immunity	Ataxia-telangiectasia
Congenital disorders	Wiskott-Aldrich syndrome
	X-linked lymphoproliferative syndrome
	Severe combined immunodeficiency
	Other immunodeficiency states
Acquired conditions of immunodeficiency	HIV infection
	Organ or stem cell transplantation
	Aging
	Chronic immunosuppressive medications
Autoimmune and rheumatologic disease	Rheumatoid arthritis
	Systemic lupus erythematosus
	Sjögren syndrome
	Celiac disease
Environmental or occupational	Herbicides
	Pesticides

cells to generate immunological diversity, and thus, specific immunity. Conditions under which malignant transformation is fostered include viral infection, chronic bacterial infection, immune deficiency, autoimmune disease, and exposure to toxins (Table 23-1). Given the degree to which the immunoglobulin genes of B cells are subjected to DNA damage in the bone marrow and germinal centers, it is not surprising that reciprocal translocations, involving an immunoglobulin gene locus and a proto-oncogene, form the hallmark of many types of B-cell lymphoma (Table 23-2).

T-cell development and biology of the T-cell lymphomas

In contrast to B-cell development, T-cell progenitors derived from common lymphoid progenitors exit the marrow and develop in the thymus. Similar to B cells, each T cell recognizes a specific antigen, but through a T-cell receptor (TCR) rather than a BCR. Like BCRs, diversity of TCRs is generated through recombination of *V*, *D*, and *J* gene segments of the four TCR genes, *alpha* (α), *beta* (β), *gamma* (γ) and *delta* (δ). Mature T cells express $\alpha\beta$ TCR or $\gamma\delta$ TCRs. Of note, $\alpha\beta$ TCRs can recognize antigens pre-

sented only in the context of a major histocompatibility complex (MHC) while $\gamma\delta$ TCRs do not have this restriction. As such, NK cells and $\gamma\delta$ T cells do not require antigen sensitization to become active and to operate as part of our innate, rather than adaptive, immune system. Meanwhile, as developing T-cells that express $\alpha\beta$ TCR mature in the thymus, their $\alpha\beta$ TCR is complexed with surface CD3 and CD4 or CD8, which identify helper and cytotoxic T-cell subsets, respectively (Figure 23-2).

The cell-of-origin approach that was so effective for categorizing B-cell lymphomas has been more difficult to apply to T-cell lymphomas due to a combination of factors including the complexity of mature T- and NK-cell lineages, with numerous functional subsets demonstrating marked phenotypic and morphologic diversity compounded by evidence of plasticity. In addition, with the noticeable exception of anaplastic lymphoma kinase-positive (ALK-positive) anaplastic large-cell lymphoma (ALCL), few recurrent cytogenetic abnormalities have been associated with mature T-cell lymphomas and, accordingly, contribute little to their categorization. Instead, clinical features and anatomic location of the disease have played major roles in defining many of the mature T- and NK-cell neoplasms included in the World Health Organization (WHO) classification, which can be grouped according to their presentation as predominantly leukemic, extranodal, or nodal disease (Table 23-3).

Diagnostic testing in lymphoproliferative disorders

Diagnosis of lymphoproliferative disorders requires some expertise and relies on a combination of morphologic findings (peripheral blood, bone marrow, or lymph node), immunophenotyping, cytogenetics, and molecular genetics.

Morphology

Well-stained peripheral blood and bone-marrow-aspirate smears provide excellent cytologic detail, facilitating evaluation of nuclear chromatin patterns and cytoplasmic coloration as well as revealing the presence of cytoplasmic inclusions and vacuoles in lymphoid cells. The degree of nuclear chromatin condensation is helpful in differentiating lymphoid blasts, which have finely granular or “open” chromatin, from mature lymphocytes, which have more opaque and condensed chromatin. Some lymphoid malignancies, such as chronic lymphocytic leukemia (CLL), have characteristic patterns of chromatin condensation, with CLL lymphocytes typically showing a “soccer ball” nuclear pattern. Likewise, Burkitt lymphoma (BL) cells can be recognized on smear preparations by their fine granular chromatin and strikingly blue, vacuolated cytoplasm.

Table 23-2 Phenotypic markers and common chromosomal translocations in selected non-Hodgkin lymphoma subtypes

NHL	slg	CD5	CD10	CD20	Other	Cyclin D1	Cytogenetics	Oncogene	Function
CLL/SLL	Weak	+	-	Dim	CD23 ⁺ , CD200 ⁺ , FMC ⁻	-	No diagnostic abnormalities*	-	-
Follicular	++	-	+	+	BCL2 ⁺ , BCL6 ⁺	-	t(14;18)	BCL2	Antiapoptosis
Mantle cell	++	+	-	+	cyclin D1 ⁺ , CD23 ⁻ , CD200 ⁻ , FMC ⁺	+	t(11;14)	Cyclin D1	Cell cycle regulator
Marginal zone/extranodal marginal zone lymphoma	+	-	-	+	-	-	t(11;18)	AP12-MALT	Resistance to <i>Helicobacter pylori</i> treatment
Lymphoplasmacytic lymphoma	++	-	-	+	CD25 ⁺⁻ , CD38 ⁺⁻	-	-	MYD88	Proliferation
Hairy cell leukemia	++	-	-	+	CD11c ⁺ , CD25 ⁺ , CD103 ⁺ , BRAF ⁺	Weak	-	BRAF	Proliferation
DLBCL	+	Rare	+/-	+	Variable	-	t(14;18), t(3;14), t(3;v)	BCL2	Antiapoptosis
							t(8;X)	BCL6	Transcription factor
								cMYC	Proliferation
								EZH2 [‡]	Histone modifier
								MYD88 [§]	Proliferation
PMBCL	-	-	-/+	+	CD30 ⁺⁻ , CD23 ⁺⁻ , PD-L1 ⁺⁻	-	t(16;X) [†]	CIITA	MHC class II transactivator
Burkitt lymphoma	+	-	+	+	BCL6 ⁺ , MYC ⁺ , TdT ⁻ , BCL2 ⁻	-	t(8;14), t(2;8), t(8;22)	cMYC	Transcription factor
								TCF3/ID3	Transcription factor and its negative inhibitor
ALCL, ALK positive	-	-	-	-	CD30 ⁺ , CD2 ⁺⁻ , CD3 ⁺⁻ , ALK ⁺ , EMA ⁺	-	t(2;5)	ALK	Tyrosine kinase
ALCL, ALK negative	-	-	-	-	CD30 ⁺ , CD2 ⁺⁻ , CD3 ⁺⁻ , ALK ⁻ , EMA ⁻	-	t(6;7)(p25.3;q32.3)	DUSP22	Phosphatase

ALCL, anaplastic large-cell lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; PMBCL, primary mediastinal large B-cell lymphoma; MALT, mucosa-associated lymphoid tissue; slg, surface immunoglobulin; SLL, small lymphocytic lymphoma; TdT, terminal deoxynucleotidyl transferase.

*A number of prognostic cytogenetic abnormalities have been identified (see Chapter 22).

†A number of partner chromosomes described.

[‡]Exclusively in GCB-like DLBCL.

[§]Exclusively in ABC-like DLBCL.

Lymph-node biopsies and bone-marrow core biopsies lack the cytologic detail of smear preparations because tissue specimens must be fixed in formalin and dehydrated, a process that shrinks the cells and obscures cytologic detail. The benefit of tissue specimens is that they provide a glimpse of the underlying architecture, a critical component in differentiating benign from malignant lymphoid proliferations and in the classification of lymphoid malig-

nancies. Lymphoid malignancies typically obliterate and “efface” underlying normal architectural features and the pattern of malignant growth, for example, nodular versus diffuse, guides subsequent classification. These patterns can be difficult to recognize in small biopsy specimens and, accordingly, needle-core biopsies of suspected lymphoid malignancies can be extremely challenging for pathologists to interpret.

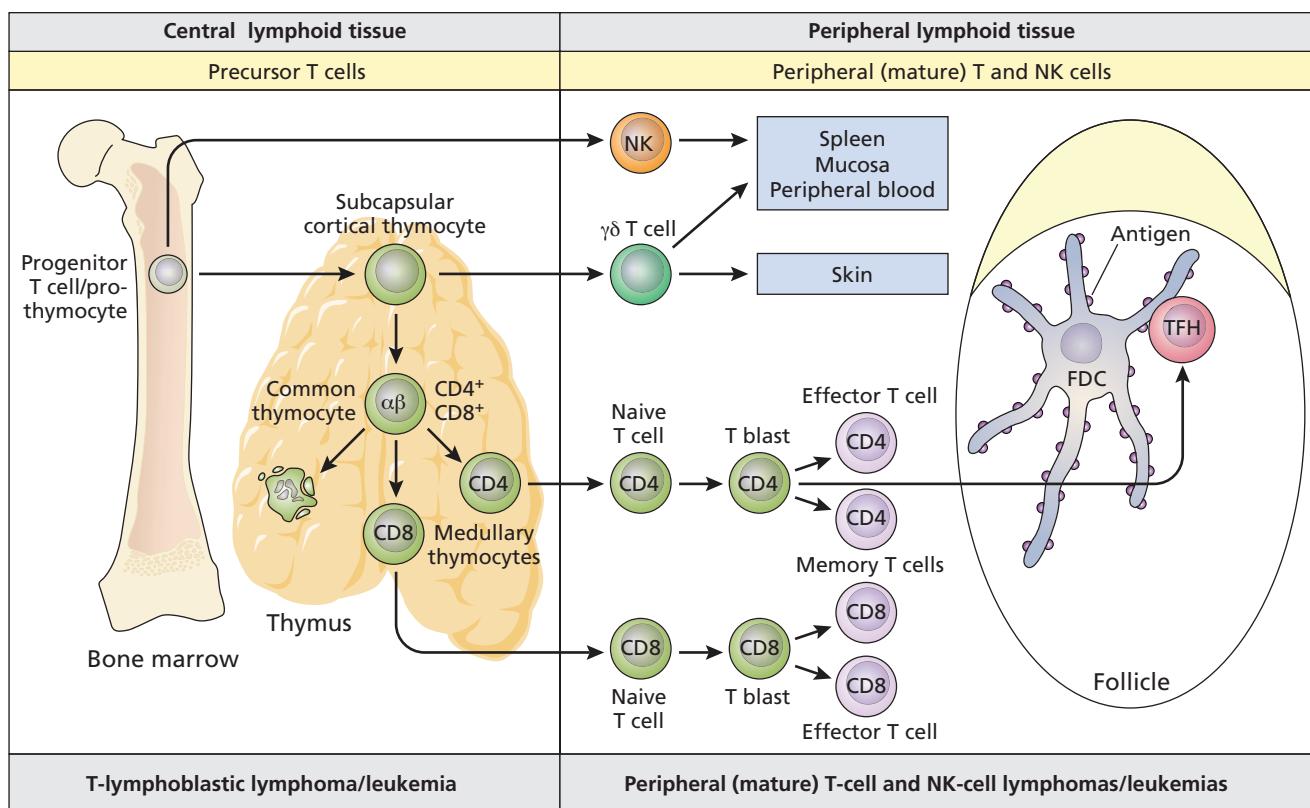


Figure 23-2 Schematic representation of T-cell differentiation (WHO 2017). FDC, follicular dendritic cells; NK, natural killer; TFH, T-helper follicular cells. Reproduced with permission from Harald Stein.

Immunophenotyping

Immunophenotyping can be performed by flow cytometry on live cells from liquid specimens or disaggregated tissue. For fixed specimens, immunophenotyping is typically performed using 3,3'-diaminobenzidine (DAB)-staining of tissue on glass slides. Immunophenotyping complements morphologic assessment by illuminating details of cell biology that would be otherwise imperceptible through the microscope. By determining cell lineage, maturation stage, and the presence of any aberrant antigen expression, immunophenotyping findings can be combined with morphologic findings to arrive at a diagnosis. For example, mantle cell lymphoma (MCL) is characterized by effacement of normal nodal architecture by small nongerminal center (CD10-negative) B cells (CD20 positive), with aberrant coexpression of CD5 (typically a T-cell marker, but expressed on a subset of B cells) and cyclin D1 (a protein that is not expressed in normal lymphocytes; its expression results from the translocation that underlies MCL). Other characteristic immunophenotypic profiles of lymphoid malignancies can be found on Table 23-2.

For B-cell malignancies, clonality can also be identified by light-chain restriction of the surface immunoglobulin. B cells normally express κ and λ light chains in a ratio of 2:1. A clonal expansion can be identified by a marked predominance of κ- or λ-expressing B cells that would not be expected in a reactive process. The immunophenotyping of T-cell neoplasms is less conclusive than for B-cell disorders because T cells lack the equivalent of light-chain restriction. Several findings can be suggestive of neoplasia, including expression of CD4 or CD8 on the majority of the T cells, lack of expression of CD4/CD8 on the majority of T cells, or coexpression of CD4 and CD8 on the majority of T cells. Often, however, molecular techniques to look at TCR gene rearrangements are necessary to differentiate reactive from clonal T-cell processes.

Molecular genetics and cytogenetics

Molecular genetic techniques can be helpful in assessing clonality when morphology and immunophenotyping are inconclusive. These techniques involve isolating the DNA from a sample and subjecting it to polymerase chain reaction (PCR) to detect rearrangements of immunoglobulin

Table 23-3 2016 World Health Organization classification of B-cell and T-cell neoplasms

B-cell neoplasms	T-cell neoplasms
Precursor B-cell neoplasms*	Precursor T-cell neoplasms*
Mature B-cell neoplasms	Mature T-cell neoplasms
B-lymphoblastic leukemia/lymphoma NOS	T-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities	
Aggressive lymphomas	Leukemic or disseminated
Diffuse large B-cell lymphoma: variants, subgroups, and subtypes/entities	T-cell large granular lymphocytic leukemia† Chronic lymphoproliferative disorders of NK cells† T-cell prolymphocytic leukemia Aggressive NK-cell leukemia Adult T-cell leukemia/lymphoma Systemic EBV-positive T-cell lymphoproliferative disorders of childhood
Diffuse large B-cell lymphoma, NOS	
Germinal center B-cell type	
Activated B-cell type	
Diffuse large B-cell lymphoma subtypes	Extranodal
T-cell/histiocyte-rich large B-cell lymphoma	Extranodal NK/T-cell lymphoma, nasal type
Primary DLBCL of the CNS	Enteropathy-type T-cell lymphoma
Primary cutaneous DLBCL, leg type	Monomorphic epitheliotrophic intestinal T-cell lymphoma
DLBCL associated with chronic inflammation	Hepatosplenic T-cell lymphoma
HHV8-positive DLBCL, NOS	Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract
EBV-positive DLBCL, NOS	Breast implant-associated anaplastic large-cell lymphoma
Other lymphomas of large B cells	Cutaneous
Primary mediastinal large B-cell lymphoma	Mycosis fungoides† Sézary syndrome†
Intravascular large B-cell lymphoma	Primary cutaneous CD30+T-cell lymphoproliferative disorder†
EBV-positive mucocutaneous ulcer	Primary cutaneous CD4+ small/medium T-cell lymphoma† Primary cutaneous acral CD8+T-cell lymphoma†
Lymphomatoid granulomatosis	Primary cutaneous anaplastic large cell lymphoma†
ALK-positive large B-cell lymphoma	Lymphomatoid papulosis
Plasmablastic lymphoma	Subcutaneous panniculitis-like T-cell lymphoma
Large B-cell lymphoma arising in HHV-8-associated multicentric Castleman disease	Primary cutaneous $\gamma\delta$ T-cell lymphoma
Primary effusion lymphoma	Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	Hydroa vacciniforme-like lymphoma
High-grade B-cell lymphoma, with <i>MYC</i> and <i>BCL2</i> and/or <i>BCL6</i> rearrangements	Nodal
High-grade B-cell lymphoma, NOS	Peripheral T-cell lymphoma, NOS
Burkitt lymphoma	Angioimmunoblastic T-cell lymphoma
Burkitt-like lymphoma with 11q aberration	Follicular T-cell lymphoma
Mantle cell lymphoma	Nodal peripheral T-cell lymphoma with TFH phenotype
In situ mantle cell neoplasia	Anaplastic large-cell lymphoma, ALK positive
	Anaplastic large-cell lymphoma, ALK negative

Table 23-3 (continued)

B-cell neoplasms	T-cell neoplasms
<i>Indolent lymphomas</i>	
Follicular lymphoma	
In situ follicular neoplasia	
Duodenal-type follicular lymphoma	
Testicular follicular lymphoma	
Pediatric-type follicular lymphoma	
Large B-cell lymphoma with <i>IRF4</i> rearrangement	
Primary cutaneous follicle center lymphoma	
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT)	
Nodal marginal zone lymphoma	
Splenic marginal zone lymphoma	
Splenic B-cell lymphoma/leukemia, unclassifiable	
Lymphoplasmacytic lymphoma	
Heavy chain disease	
Plasma cell neoplasms	
CLL/SLL	
Monoclonal B-cell lymphocytosis	
B-cell prolymphocytic leukemia	
Hairy cell leukemia	

*All precursor neoplasms are considered aggressive.

†Indolent T-cell neoplasms, all other T-cell neoplasms are considered aggressive.

CLL, chronic lymphocytic leukemia; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; NK, natural killer; SLL, small lymphocytic lymphoma.

or TCR genes. The demonstration of a dominant rearrangement of the immunoglobulin or TCR genes is indicative of a clonal process.

Chromosomal translocations are common in lymphoproliferative disorders and may contribute to the transformation process or cellular proliferation (Table 23-2). Commercial probes are available for detection of most translocations by fluorescent in situ hybridization (FISH) and can be useful markers of malignancy and for identifying specific lymphoma subtypes. Use of microarray technology has defined gene-expression profiles of various lymphoid malignancies and compared them to normal lymphoid populations. This technique has been successfully applied to a number of B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL), FL, CLL, and MCL, to identify expression patterns that correlate with patient outcome. However, technical difficulty with assessing gene-expression profiles in the clinical laboratory, especially in formalin-fixed tissues, has hampered clinical application of these findings. Despite this, pathologists and oncologists have managed to apply the

DLBCL gene-expression discoveries to the clinical realm by utilizing surrogate immunohistochemistry-based expression panels to differentiate the better-prognosis germinal-center B-cell-like DLBCL from the poor-prognosis activated B-cell-like DLBCL. More recently, next-generation-sequencing (NGS) technology has been utilized to deeply interrogate the genomes of various lymphoid malignancies. While many such studies are still ongoing, landmark discoveries of single causative mutations of *BRAFV600E* in hairy cell leukemia (HCL) and *MYD88 L265P* in Waldenström macroglobulinemia have thus far been reported (Table 23-2).

Assessment of lymphoma genetics via cell-free DNA (cfDNA) is an emerging analytic technique that has shown promise in assessing tumor kinetics, detecting occult disease, and assessing depth of response to therapy. This technique involves sequencing small fragments of cell-free DNA shed by apoptotic tumor cells into peripheral blood. Analysis of cfDNA ostensibly generates a more comprehensive assessment of tumor heterogeneity compared to tissue biopsy and facilitates serial monitoring of tumor genetics simply

by phlebotomy. For patients with B-cell lymphoma, sequencing cell-free immunoglobulin receptor (VDJ) gene sequences by NGS can identify and quantify tumor-specific rearrangements thereby facilitating assessment of tumor kinetics during therapy as well as depth of response. The kinetics and clearance of tumor cfDNA in patients with DLBCL have been associated with prolonged progression-free survival. Likewise, assessment of lymphoma-relevant mutations other than immunoglobulin receptor genes by ultra-deep sequencing of cfDNA can also be performed and clinical response in patients with DLBCL treated with R-CHOP found to be associated with clearance of cfDNA basal mutations in the peripheral blood.

Classification of non-Hodgkin lymphomas

The classification of lymphoproliferative disorders continues to evolve as our understanding of the biology of these diseases progresses. The current classification system used is the *World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues*, which was updated in 2017 (Table 23-3) and incorporates the explosion of new clinical, pathological, and genetic/molecular information that occurred since the previous 2008 publication. The B- and T-cell neoplasms are separated into precursor (lymphoblastic) neoplasms and mature B- or T-cell neoplasms. Overall, ~90% of all non-Hodgkin lymphomas (NHLs) in Western countries are of mature B-cell origin, with DLBCL and FL being the most common subtypes. In children, Hodgkin lymphoma (HL) is more predominant, and the aggressive NHLs of lymphoblastic lymphoma and BL are much more commonly encountered than are indolent neoplasms. The incidence of NHL is lower among Asian populations, in whom T-/NK-cell neoplasms are more frequent.

While the premise of the WHO classification is to separate lymphoid malignancies into distinct, nonoverlapping entities, it also recognizes that the biology of particular tumors crosses the boundaries between current categories. The classification of these gray-zone malignancies have been updated in the 2017 WHO monograph. “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma” remains unchanged, whereas “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma” has been eliminated and replaced by “high-grade B-cell lymphoma, NOS (where NOS stands for “not otherwise specified”) and “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.” Common gene expression and epigenetic profiles between primary mediastinal large B-cell lymphoma and classical Hodgkin lymphoma (cHL) indicate a true biologic gray

zone between these two entities exists. Likewise, certain cases of DLBCL have been found to have expression profiles of BL, although these cases differed clinically and genetically from classic BL and vice versa. Biologically, many of these cases may lie in the gray zone because they have rearrangements in both *cMYC* and *BCL2* or *BCL6* genes (“double-hit” lymphomas) and are more clinically aggressive than standard DLBCLs, hence their revised classification as “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.” The remaining cases that exist in the boundary between BL and DLBCL without *MYC* and *BCL2* or *BCL6* rearrangements are now classified as “high-grade B-cell lymphoma, NOS.”

For clinical purposes, the NHLs can be broadly separated into indolent or aggressive categories (Table 23-3). *Indolent lymphomas* generally are incurable with most standard therapeutic approaches and are typified by a chronic course with repeated relapses and progression with standard therapy. Some of these patients, however, survive many years with remarkably stable disease even in the absence of specific therapy. Median survival is measured in decades, and the majority of patients live a normal life expectancy compared to age-matched controls, thanks to the efficacy of modern therapy. Most, but not all, *aggressive lymphomas* are potentially curable with combination chemotherapy. Aggressive subtypes usually have a more acute presentation, often with B-symptoms, and a more rapid progression than the indolent entities. In the event of failure to achieve complete remission (CR) following treatment or with relapse after an initial therapeutic response, survival usually is measured in months rather than years. Some of these patients, however, are cured by second-line chemotherapy and stem-cell transplantation approaches as described later in this chapter.

Epidemiology, pathogenesis, and molecular characterization

Data from cancer registries show that the incidence of NHL has been increasing steadily in North America and other industrial countries with a doubling of cases between 1970 and 1990 and stabilization thereafter. In 2019, there will be an estimated 74,200 new cases of NHL, representing 4–5% of all cancer diagnoses among men and women, and 19,970 deaths. The reasons for this increasing incidence are unknown but are the subject of ongoing epidemiologic investigations. Associations have been made with occupational exposure to certain pesticides and herbicides (Table 23-1). Agricultural workers with cutaneous exposure to these agents have a 2- to 6-fold increased incidence of NHL, possibly contributing to the relatively greater frequency of lymphoma in rural vs urban populations.

Risk factors may differ between developing B- and T-cell lymphomas. A large epidemiologic study from the International Lymphoma Epidemiology Consortium (Inter-Lymph) identified eczema, T-cell activating autoimmune diseases, a family history of myeloma, and occupation as a painter as increasing the risk for T-cell lymphoma. A history of B-cell-activating autoimmune disease and hepatitis C seropositivity were associated with increased risk for certain B-cell lymphomas.

Immunosuppression associated with HIV infection or iatrogenically induced immune suppression in the organ transplantation setting is associated with an increased incidence of aggressive B-cell lymphomas, likely due to dysregulated B-cell proliferation and susceptibility to viruses, such as Epstein-Barr virus (EBV) (Table 23-1). In children, the incidence of NHL is increased in several disorders that have immunodeficiency from primary immune disorders, including ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable or severe combined immunodeficiency, and X-linked lymphoproliferative disorder.

Infection with the bacterium *Helicobacter pylori* is strongly associated with gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Table 23-1). Patients with MALT limited to the stomach often achieve CR after successful therapy to eradicate *H pylori*, indicating that the lymphoma remains dependent in part on continued antigenic drive from the microorganism. Associations have also been made between orbital infection by *Chlamydophila psittaci* and orbital adnexal MALT lymphoma, infection with *Campylobacter jejuni* and immunoproliferative small intestinal disease, and *Borrelia burgdorferi* or *Borrelia afzelii* and cutaneous MALT lymphoma. These intriguing associations need to be firmly established by additional investigation. Response to antimicrobial therapy among MALT lymphomas driven by infectious pathogens has been highly variable. The majority of gastric MALT lymphomas respond to *H. pylori* directed antibiotic treatment, while response of ocular adnexal or cutaneous MALT lymphomas to *Chlamydophila* or *Borrelia* directed therapies, respectively, has been unsuccessful overall, with some geographic variability.

Certain viral infections have been linked with specific subtypes of NHL. EBV has a clear pathogenic role in endemic, as well as in some cases of sporadic, BL and in many cases of HIV-related aggressive B-cell lymphoma and discrete subtypes of B-cell and T-cell lymphomas. EBV-positive DLBCL NOS is thought to be associated with age-related immunosuppression. EBV is strongly associated with extranodal T-/NK-cell lymphoma, nasal type, which is seen most commonly in Asia and in Central and South America. EBV is also detected in 70% to 80% of cases of angioimmunoblastic T-cell lymphoma (AITL). The gammaherpesvirus

human herpesvirus 8 (HHV-8, also called Kaposi sarcoma-associated herpesvirus [KSHV]), first described in Kaposi sarcoma but also associated with an unusual primary body cavity lymphoma (primary effusion lymphoma), is most commonly seen in patients with AIDS. HHV-8 also has been described in association with multicentric Castleman disease. The retrovirus human T-cell lymphotropic virus 1 (HTLV-1) is associated with adult T-cell leukemia/lymphoma endemic to Japan, central Africa, and the Caribbean. Chronic hepatitis C virus infection has been linked to the development of B-cell NHL, particularly marginal-zone lymphoma and DLBCL, possibly via chronic BCR stimulation through direct binding of a viral envelope protein.

Specific chromosomal translocations are strongly associated with individual subtypes of B-cell NHL (Table 23-2). The majority of these arise early in B-cell differentiation, during the process of immunoglobulin gene rearrangement, when errant fusion of immunoglobulin promoter and enhancer elements with other genes leads to dysregulated oncogene expression. Careful study of such translocations has provided important insights into pathogenic mechanisms in lymphoma. The most frequent of these translocations are: (i) t(14;18), with resultant overexpression of the anti-apoptotic gene *BCL2*, which is present in ~85% of FLs; (ii) t(11;14) with cyclin D1 overexpression, which is present in virtually all MCLs; and (iii) t(8;14), t(2;8), and t(8;22) of BL, which fuse an immunoglobulin heavy- or light-chain gene promoter to the cMYC transcription factor. *BCL6*, a chromosome-3 transcription-factor gene capable of promiscuous rearrangement with multiple translocation partners, is most commonly identified in DLBCL. The t(2;5) (p23;q35) fuses the *ALK* gene with nucleophosmin and is found in a subset of ALCL. Several other translocation partners with the *ALK* gene also have been described in this disease. This translocation and *ALK* expression are associated with a more favorable prognosis in ALCL (see also the section Peripheral T-cell lymphomas in this chapter). Among ALCL patients without an *ALK* rearrangement, DUSP22 translocations have been found in a subset of cases and predict a favorable prognosis.

Gene expression profiling has defined molecular signatures in lymphoma that have been utilized to identify prognostically significant disease subsets in DLBCL, FL, MCL, CLL, and T-cell ALCL as well as illuminating the existence of gray-zone lymphomas that lie between DLBCL and BL, as well as DLBCL and cHL. More recently, next-generation sequencing has provided some early insight into the mutational landscape of several lymphomas including the previously mentioned single causative mutations of *BRAFV600E* in HCL and *MYD88 L265P* in Waldenström macroglobulinemia. Additionally, the mutational landscape

of GCB-like DLBCL has been found to be distinct from ABC-like DLBCL, with GCB-like DLBCL harboring an activating *EZH2* mutation in a subset of cases, while ABC-like DLBCL may harbor activating *MYD88* and *CD79B* mutations. These discoveries continue to refine lymphoma classification and elucidate novel therapeutic targets.

Staging and prognostic factors

Staging procedures generally include careful physical examination for lymphadenopathy and organomegaly; computed tomography (CT) scans of the neck, chest, abdomen, and pelvis; fluorodeoxyglucose positron emission tomography (FDG-PET) imaging; and may require bone marrow biopsy. CT or magnetic resonance imaging (MRI) of the brain and evaluation of the cerebrospinal fluid are indicated in patients with BL or lymphoblastic lymphomas and should be considered in patients with DLBCL involving high-risk sites, including the paranasal sinuses or testes. The Ann Arbor staging system, identifying patients as having stage I (localized) to stage IV (extensive extranodal) disease, originally was devised for use in HL but was later adopted for use in NHL. Patients are further stratified as to the absence (A) or presence (B) of systemic symptoms, namely, fevers, drenching night sweats, or unintentional weight loss of 10% or more within 6 months of diagnosis. Several limitations become apparent when the Ann Arbor classification is applied to NHL and, as a result, a revised staging system, called the Lugano classification, was proposed in 2014 (Table 23-4). Patients with Ann Arbor stage I or II disease can be grouped and considered as having “limited stage” disease whereas patients with Ann Arbor stage III or IV disease can be grouped and considered as having “advanced stage” disease. Other recommendations from the Lugano classification include the following: (i) consider FDG-PET/CT as standard imaging for FDG avid lymphomas but employ CT for non FDG-avid histologies; (ii) reserve the suffix A or B only for HL; (iii) eliminate the X designation for bulky disease (because there is no universal definition for

bulk) and replace it with a recording of the largest nodal diameter; and (iv) eliminate the need for staging bone-marrow biopsies in aggressive NHL histologies if a PET-CT scan was used for staging.

Lymphoma staging has only limited prognostic usefulness. To more fully incorporate additional relevant prognostic features, models have been developed in multiple NHL subtypes, including DLBCL, FL, and MCL. The most widely used clinical prognostic model for stratifying patients with aggressive NHLs is the International Prognostic Index (IPI). The purpose was to identify pretreatment variables that predict relapse-free and overall survival (OS) in patients treated with doxorubicin-containing combination chemotherapy. The following five risk factors were found to be independently associated with clinical outcome and may be referred to by the mnemonic *APLES*: (i) age older than 60 years, (ii) ECOG PS > 1, (iii) elevated serum lactate dehydrogenase (LDH), (iv) number of extranodal sites of disease > 1, and (v) stage III or IV. The IPI score is derived as a simple additive score from 0–5, has been widely adopted to estimate prognosis in patients with NHL, and is useful in some of the other lymphoma subtypes. Of note, these survival estimates were established before the use of rituximab for diffuse large B-cell lymphoma.

Limited studies support that the IPI is still prognostic in the rituximab-treatment era. A revised IPI (R-IPI), based on data from the British Columbia Cancer Agency, may define new risk groups in rituximab-treated patients: very good risk (0 risk factors, 4-year progression-free survival [PFS] 90%); good risk (1, 2 risk factors, 4-year PFS 70%); and poor risk (>2 risk factors, 4-year PFS 50%). The Deutsche Studiengruppe für Hochmaligne Non-Hodgkin-Lymphome (DSHNHL) group also evaluated the usefulness of the IPI in over 1,000 patients enrolled on prospective clinical trials and found that IPI did effectively separate patients into the previously established risk categories with 3-year PFS ranging from 56% in the highest risk patients to 87% in the lowest risk (Table 23-5).

Table 23-4 Lugano staging system for NHL

Stage		Involvement	Extranodal (E) status
Lugano	Ann Arbor		
Limited	I	One node or a group of adjacent nodes	Single extranodal lesion without nodal involvement
Limited	II	Two or more lymph node regions on the same side of the diaphragm	Stage II by nodal extent with limited contiguous extranodal extension
Advanced	III	Involvement of lymph node regions on both sides of the diaphragm, nodes above the diaphragm with or spleen involvement	Stage III by nodal extent with limited contiguous extranodal extension
Advanced	IV	Additional noncontiguous extralymphatic involvement	Not applicable

Table 23-5 The IPI in DLBCL in the rituximab era

Risk factors*	3-year PFS	3-year OS
0, 1	87%	91%
2	74%	81%
3	59%	65%
4, 5	56%	59%

*IPI risk factors are age \geq 60 years, abnormal LDH, PS \geq 2, stage III or IV, and >1 extranodal sites.

Although the IPI scoring system provides useful prognostic information, there is no definitive evidence that outcome is altered by using intensive regimens in high-risk patients. Numerous studies have been reported and others are still in progress that assess the utility of the IPI and “risk-adjusted” or “risk-adapted” therapeutic strategies. These include trials of high-dose therapy (HDT) and autologous stem-cell transplantation (ASCT) for aggressive lymphoma patients with high IPI scores; however, such strategies currently are not routinely recommended because standard approaches are effective in the majority of patients, and the value of HDT has only been suggested in underpowered subset analyses of larger clinical trials showing no statistical benefit for this approach in the overall patient population (see the section “Diffuse large B-cell lymphoma” later in this chapter). The IPI is useful in comparing studies and also in the investigation of new prognostic factors to determine the independent effect on outcome.

The IPI score is predictive of survival in indolent lymphomas, namely, FL, although using the IPI, the majority of these patients fall into the low-risk or low-intermediate-risk categories. As such, a new index was developed specifically for FL, called the Follicular Lymphoma International Prognostic Index (FLIPI), in hopes of better stratifying patients (Table 23-6). This index can be remembered by the mnemonic No-LASH. The five clinical factors that are the strongest predictors of outcome in multivariate analysis were: (i) number (no.) of nodal sites of disease (>4), (ii) elevated LDH, (iii) age older than 60 years, (iv) stage III or IV disease, and (v) hemoglobin <12 g/dL. Compared with the IPI, the FLIPI provides a better distribution of patients across the risk categories of low risk (0 to 1 factor), intermediate risk (2 factors), or high risk (>2 factors). The 10-year OS rates were 71% (low risk), 51% (intermediate risk), and 36% (high risk), respectively (Table 23-6). Similarly, an international prognostic index for MCL (the Mantle Cell Lymphoma International Prognostic Index [MIPI]) also has been developed and incorporates age, performance status (PS), LDH, and white blood cell (WBC) level (Table 23-7).

Role of FDG-PET imaging

FDG-PET scanning is useful both for staging and for assessing response to lymphoma therapy and is generally recommended as part of routine staging and end-of-treatment response assessment in FDG-avid lymphomas. The 5-point scale (Deauville criteria [Table 23-8]) should be used for PET interpretation, and scores of 1 to 3 at completion of

Table 23-6 The Follicular Lymphoma International Prognostic Index (FLIPI)

Risk model and group	No. of factors	Distribution of cases (%)	5-year OS (%)	10-year OS (%)
FLIPI*				
Low	0–1	36	91	71
Intermediate	2	37	78	51
High	³ 3	27	53	36

*FLIPI risk factors: No-Lash, number of nodal sites of disease (>4); elevated LDH, age >60 years, stage III or IV disease, and hemoglobin ≤ 12 g/L.

Table 23-7 The Mantle Cell Lymphoma International Prognostic Index (MIPI)

Points	Age, years	ECOG PS	LDH/ULN	WBC, cells/mm ³
0	<50	0–1	≤ 0.67	$<6,700$
1	50–59	—	0.67–0.99	6,700–9,999
2	60–69	2–4	1.00–1.49	10,000–14,999
3	³ 70	—	≥ 1.50	$\geq 5,000$

MIPI risk factors are age, PS, LDH, WBC level.

Formula for MIPI: $[0.03535 \times \text{age (years)}] + 0.6978$ (if ECOG >1) $+ [1.367 \times \log_{10}(\text{LDH}/\text{ULN})] + [\log_{10}(\text{WBC count})]$.

Simplified MIPI: low risk, 0–3 points; intermediate risk, 4–5 points; high risk, 6–11 points.

ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; PS, performance status; ULN, upper limit of normal; WBC, white blood cell.

Table 23-8 Deauville 5-point scale for PET interpretation in lymphoma

Score	Visual description
1	No uptake
2	Uptake \leq mediastinum
3	Uptake $>$ mediastinum but less than liver
4	Uptake moderately higher than liver
5	Uptake markedly higher than liver

therapy are considered consistent with complete remission, regardless of the size of any residual masses. Some studies indicate that interim PET scanning, performed mid-treatment, can identify patients at higher risk for treatment failure; however, it is unknown whether therapy should be altered based upon the results of a mid-treatment PET scan. False-positive results can occur in the setting of inflammation, granulomatous disease, and infection, and a biopsy should be performed in a PET-positive patient in remission by CT scan if high-dose chemotherapy and stem-cell transplantation (HDC/SCT) are under consideration.

Patient management and follow-up

With over 60 lymphoma subtypes, detailed management guidelines for each subtype and disease stage are beyond the scope of this chapter. The reader is encouraged to refer to the NCCN guidelines at <http://www.nccn.org/> which is an outstanding resource for the treating clinician.

Patient surveillance following treatment of lymphoma should address both long-term complications of therapy and disease recurrence. Long-term effects of therapy depend on the type of treatment and whether radiotherapy was also administered. For example, radiotherapy to the head and neck region leads to decreased salivation with dental caries, and if the thyroid is included in the radiation field, a large proportion of patients eventually will become hypothyroid. Women who have had mantle radiation should receive a mammogram beginning 10 years after radiation or at age 40 years, whichever comes first. In younger women, MRI breast imaging also can be considered, given the reduced sensitivity of mammography in this population.

Long-term survivors are at risk of second malignancies, which are dependent on the treatment administered. For example, radiated patients are at risk for carcinomas and sarcomas in the radiated field, while those who have had alkylating agents are at risk for therapy-related myelodysplastic syndrome or acute myeloid leukemia. Once primary therapy has been completed and remission is documented, patients typically are followed every 3 months for the first 2 years, then every 6 months until 5 years, and then annually thereafter.

Most recurrences of aggressive lymphoma occur in the first 2 years after treatment, although late relapses beyond 5 years do occur in a minority of patients. Patients with indolent lymphoma have a lifelong risk of relapse and typically are seen every 3 months for the first 2 years and then every 6 to 12 months indefinitely. There is no evidence that routine CT or PET imaging affects outcome of patients, and newer guidelines recommend minimizing surveillance imaging in indolent lymphomas and discourage any minimal use of surveillance imaging in aggressive lymphoma.

KEY POINTS

- NHLs are biologically and clinically heterogeneous; accurate diagnosis by a hematopathologist using the WHO classification is essential for optimal management.
- The majority of NHLs are of B-cell origin and are categorized broadly as indolent vs aggressive subtypes.
- The incidence of NHL is increasing in Western countries.
- Specific chromosomal translocations are associated with specific subtypes of lymphoma and are pathogenetically involved in malignant transformation and progression.
- The IPI score provides important prognostic information for outcome and survival in aggressive lymphomas. The FLIPI has been developed specifically for FL.

Indolent B-cell NHL

The indolent B-cell lymphomas include the histologies shown in Table 23-3, and the most commonly encountered subtype is FL, which accounts for 20% to 30% of all lymphomas. Other subtypes include marginal-zone lymphomas (nodal, splenic, and extranodal [MALT] types) and lymphoplasmacytic lymphoma. This category also includes CLL/SLL, which is discussed in Chapter 24.

CLINICAL CASE

A 53-year-old man is diagnosed with stage IV FL after noticing a lump on his neck while shaving. A biopsy reveals a lymph node with enlarged, closely packed follicles with distorted architecture. Inside the follicles are small lymphocytes with irregular nuclei. The cells stain positive for CD20, CD10, and BCL2. The staging evaluation reveals widespread lymphadenopathy, involving five nodal groups, with the largest node measuring just over 3 cm. The hemoglobin and LDH are normal. He has no disease-related symptoms and his Eastern Cooperative Oncology Group (ECOG) PS is 0. The FLIPI score is 2, and he has a low tumor burden by Groupe d'Etude des Lymphomes Folliculaires (GELF) criteria.

Follicular lymphoma

FL is the prototypical and most common indolent lymphoma, with about 15,000 new cases diagnosed each year in the United States. Although incurable, the prognosis is quite good and has substantially improved in the modern era with the majority of patients now predicted to have a normal life expectancy compared to age-matched controls.

FLs are derived from germinal-center B cells and are graded based on the number of centroblasts per high-power field: grade 1-2 (0-15), and grade 3 (>15). Grade 3 is further classified into grade 3A (centrocytes present) and grade 3B (solid sheets of centroblasts). Grade 1-2 constitutes the typical low-grade follicular lymphoma, while grade 3 FL is relatively uncommon (<20% of all FLs); the natural history of this entity is less clear but may behave more aggressively. Most contemporary clinical trials will allow grade 3A to be included with grade 1-2 cases, whereas grade 3B is excluded and managed akin to DLBCL. Immunophenotypically, FL cells are CD20⁺, CD10⁺, BCL6⁺, BCL2⁺, and CD5⁻. Up to 90% of cases have a t(14;18) with a higher frequency observed in grade 1-2 FLs.

The 2016 WHO classification has identified several variants of FL. These include in-situ follicular neoplasia, duodenal-type follicular lymphoma, and testicular follicular lymphoma; alongside three separately classified indolent B-cell lymphomas of follicle-center origin, primary cutaneous follicle-center lymphoma, pediatric-type follicular lymphoma and large B-cell lymphoma with *IRF4* rearrangement. “In situ follicular neoplasia” replaced the previous diagnosis of “in situ follicular lymphoma,” consistent with growing conservatism in diagnosis of lymphoid neoplasia with a low rate of progression. Both duodenal-type and testicular follicular lymphomas are localized, biologically distinct, extranodal variants of FL that have excellent long-term outcomes with watch-and-wait approaches after surgical excision.

Primary cutaneous follicle-center lymphoma should be distinguished from FL. It is derived from follicle-center cells and can have a follicular, follicular and diffuse, or diffuse growth pattern. Unlike nodal FL, the neoplastic cells are usually BCL-2 negative and typically occur as solitary or localized skin lesions on the scalp, forehead, or trunk; only 15% present with multi-focal lesions. The clinical course is usually very indolent and can be managed with low-dose radiation and other site-directed approaches.

Likewise, pediatric-type follicular lymphoma and large B-cell lymphoma with *IRF4* rearrangement are distinguished from FL in the 2016 WHO. As the name suggests, pediatric-type FL typically occurs in children and young adults and is a nodal disease characterized by large

expansile highly proliferative follicles comprised of blas-toid cells that lack the typical t(14;18) translocation and are BCL2 negative. Despite the aggressive cytologic features, the prognosis is excellent with nearly all cases presenting with localized disease that may not require treatment other than excision. Large B-cell lymphoma with *IRF4* rearrangement also typically occurs in children and young adults, involving Waldeyer ring or cervical lymph nodes, with a follicular or diffuse pattern of intermediate-to-large follicle-center B cells that aberrantly coexpress the post-germinal-center protein IRF4/MUM1. In contrast to pediatric-type follicular lymphoma, patients with large B-cell lymphoma with *IRF4* rearrangement typically require combination immunochemotherapy with or without local radiation.

Management of localized follicular lymphoma

Limited-stage (Ann Arbor I or II) FL is relatively uncommon and, as a result, there are no randomized studies indicating the optimal management strategy. Rather, most of the data are observational. Older studies suggested a proportion of patients might be cured with external beam radiation. MacManus and Hoppe (1996) found that ~40% of limited-stage patients with FL remained disease-free at 10 years after radiation treatment; late relapses beyond 10 years were unusual. Other studies also reported a 10-year disease-free survival (DFS) rate of ~40% to 50%, suggesting that cure is possible with this approach in a proportion of patients. Given the excellent long-term outcomes for patients with localized FL, there is concern for late-onset radiation-induced complications, including second primary cancers. Recent data indicate that radiation fields can be reduced without adversely impacting disease control. As a result, contemporary strategies tend to utilize an involved-site approach. Studies evaluating chemotherapy plus radiation (combined modality therapy [CMT]) have demonstrated improved PFS without an obvious effect on OS. Therefore, the CMT approach is likely best reserved for the rare patient who presents with bulky (node >7 cm) limited-stage FL. Finally, an alternative management strategy for this patient population is surveillance alone. A Stanford report of stage I and II patients, who received no initial therapy, showed that more than half of the 43 patients did not require therapy at a median of 6 years and that 85% of patients were alive at 10 years. A report from a large observational database found that the following treatment approaches were utilized for 471 stage I FL patients: rituximab combined with chemotherapy 28%, radiation therapy (XRT) 27%, observation 17%, CMT 13%, rituximab 12%, and other 3%. Approaches utilizing systemic therapy produced better PFS outcomes than XRT alone, but there were no OS differences between any of

the approaches; therefore optimal management should be personalized for the patient.

Approach to patients with advanced-stage follicular lymphoma

Patients with advanced-stage FL are considered incurable with standard chemotherapy. The disease generally is responsive to treatment, however, and there are numerous effective treatment options. As a result, the prognosis is excellent relative to other cancers. A typical patient undergoes a number of different treatments, often separated by several years, and the goal of management is to achieve a normal life expectancy. Advanced-stage FL can be thought of as a chronic disease that requires long-term management, and the management is largely a matter of determining how to sequence the different therapies.

The approach to a newly diagnosed patient needs to be individualized, factoring in the presence or absence of symptoms, tumor burden, patient age and comorbidities, and goals of therapy. A 2×2 table can be constructed to help with the initial approach of separating patients by symptoms and tumor burden (Table 23-9). Using this approach, four patient categories are generated: (i) asymptomatic, low tumor burden; (ii) asymptomatic, high tumor burden; (iii) symptomatic, low tumor burden; and (iv) symptomatic, high tumor burden. Patients with asymptomatic, low tumor burden should be followed with surveillance alone. Patients with asymptomatic, high-tumor-burden FL should generally start therapy soon after diagnosis, although selected patients may be observed initially, such as the very elderly or those who just meet the high-tumor-burden criteria (eg, three nodes in the 3- to 4-cm range). Patients with symptomatic, low-tumor-burden disease do benefit from therapy, often with mild treatment approaches including rituximab alone or low-dose radiation. From a decision-making standpoint, patients with symptomatic, high-tumor-burden FL are the most straightforward. They require treat-

ment, typically with chemoimmunotherapy, although there is little consensus on which specific chemoimmunotherapy regimen is best.

Management of asymptomatic, low-tumor-burden follicular lymphoma

Asymptomatic patients may be candidates for a strategy of surveillance alone. To determine whether observation is an option, one should assess the tumor burden. The GELF criteria (Table 23-10) are the most commonly used criteria to assess tumor burden and to assess eligibility for clinical trials. The surveillance strategy was first advocated at Stanford University when two retrospective studies suggested no detriment in patient outcome. Three randomized clinical trials in the pre-rituximab era later confirmed that low-tumor-burden FL patients assigned to surveillance alone experienced the same OS compared with patients assigned immediately to treatment. The median time to first chemotherapy in all studies was 2.3–3 years. More recently, a randomized trial compared surveillance alone with single-agent rituximab in patients with previously untreated, asymptomatic, low-tumor-burden FL. Patients were assigned to surveillance (arm A), rituximab at 4 weekly doses (arm B), or rituximab at 4 weekly doses plus a single dose every 2 months for 2 years (arm C). A significant prolongation in PFS and prolongation in the time to first chemotherapy was observed for the patients randomized to rituximab; however, there was no difference in OS at 3 years (95% in all arms), consistent with randomized trials in the pre-rituximab era. The study also evaluated quality of life (QOL). Given that these patients are symptom free, the main QOL issues tend to be anxiety, depression, and adjustment to illness. The study found that anxiety and depression were more common in patients with low-tumor-burden FL than in the general population but were still relatively infrequent at 13% and 3%, respectively. Patients in all treatment arms adapted to their illness over time. The

Table 23-9 Algorithm for the approach to the newly diagnosed FL patient

	Low tumor burden	High tumor burden
Symptoms absent	Surveillance	R-chemotherapy +/-MR
Symptoms present	Single-agent rituximab, low dose radiation to single symptomatic site of disease, or R-chemotherapy	or O-chemotherapy +/-MR or rituximab monotherapy or surveillance in older/less fit patients

R, rituximab; MR, maintenance rituximab; O, obinutuzumab; MO, maintenance obinutuzumab.

Table 23-10 GELF criteria for high tumor burden

Any nodal or extranodal mass >7 cm
Three or more nodal sites with diameter of >3 cm
Elevated LDH
Hb <10 g/dL, ANC <1.5 × 10 ⁹ /L, Plts <100 × 10 ⁹
Spleen >16 cm by CT scan
Risk or organ compression or compromise
Significant serous effusions

Meeting any one criterion qualifies as high tumor burden. All must be absent to qualify as low tumor burden.

ANC, absolute neutrophil count; GELF, Groupe d'Etude des Lymphomes Folliculaires; Hb, hemoglobin; LDH, lactate dehydrogenase; Plts, platelets.

patients identified as “anxious” adapted more readily when assigned to rituximab treatments. It is reasonable to conclude that, given no OS difference observed to date, surveillance remains the appropriate standard for the asymptomatic, low-tumor-burden FL population, though rituximab monotherapy can be considered in selected patients.

If administering single-agent rituximab to a patient with low-tumor-burden FL, should one utilize a maintenance strategy or simply retreat at progression? This dosing question was addressed in the RESORT study. After induction therapy with single-agent rituximab, patients with low-tumor-burden indolent B-cell NHL were randomized to receive maintenance rituximab once every 3 months until treatment failure or to be periodically retreated with rituximab (retreated with 4 weekly doses at each progression) until treatment failure. The trial revealed no difference in the time-to-treatment failure between the two dosing strategies. Patients on the maintenance arm, however, utilized four times as much rituximab. There was no difference in quality of life, depression, or anxiety between the two strategies. Based on these results, a retreatment strategy is preferred if opting for single-agent rituximab in this patient population.

Therapy for symptomatic and/or high-tumor-burden follicular lymphoma

Treatment is indicated for FL when patients develop adverse symptoms related to their disease, or develop bulky disease which is at high risk for causing symptoms or obstruction in the near future. The addition of rituximab to conventional chemotherapy has improved outcomes in FL, including response rates, PFS, event-free survival (EFS), and OS. Table 23-11 summarizes major studies combining rituximab with chemotherapy.

Clearly, rituximab added to chemotherapy is a therapeutic advance in FL, though the optimal chemotherapy backbone remains unsettled. Data generated prior to the introduction of bendamustine in the US indicated the most commonly used regimens in the United States were R-CHOP (rituximab, cyclophosphamide, vincristine,

prednisone) (60%), R-CVP (rituximab, cyclophosphamide, prednisone) (27%), and R- fludarabine-based (13%). A randomized comparison of these regimens indicated R-CHOP had the best risk-benefit profile because it was more active than R-CVP and less toxic than R-FM. Subsequently, however, bendamustine, an alkylating agent with nucleoside-analogue properties, gained widespread adoption as the chemotherapy platform of choice in FL. A phase 3 trial comparing bendamustine plus rituximab (BR) to R-CHOP demonstrated better efficacy and reduced toxicity with BR. In this multicenter phase 3 study, 549 patients with high-tumor-burden indolent NHL and MCL (median age 64 years) were randomized to receive bendamustine 90 mg/m² on days 1 and 2, with rituximab 375 mg/m² on day 1, every 28 days (the BR group) or to receive standard R-CHOP chemotherapy every 21 days (the R-CHOP group). The overall response rates (ORRs) were similar in the BR and R-CHOP groups (92.7% vs 91.3%, respectively), but the CR rate was significantly higher in the BR group (39.8%) compared with the R-CHOP group (30.0%) ($P=.03$). When evaluating just the FL patients, with a median follow-up of 45 months, the median PFS was significantly longer in the BR group compared with R-CHOP group (median PFS, not reached vs 40.9 months, $P=.007$). OS did not differ between both groups. There was less hematologic toxicity, alopecia, infections, peripheral neuropathy, and stomatitis with BR. Drug-associated erythematous skin reactions were seen more frequently in the BR group. These data suggest that BR is a better option for untreated high-tumor-burden FL.

A confirmatory randomized phase 3 trial (BRIGHT study) was conducted in North America. Previously untreated indolent NHL patients with high tumor burden were randomized to BR or R-CHOP/R-CVP. Control arm patients were identified as R-CHOP or R-CVP candidates prior to randomization. The primary endpoint was to show noninferiority of BR in the CR rate. Seventy percent of the 447 enrolled patients had FL, and, in these

Table 23-11 Randomized trials of chemotherapy versus R-chemotherapy in high tumor burden, advanced-stage follicular lymphoma

Study	Treatment	N	Median follow-up	ORR	Time to event	OS
Hiddemann et al, <i>Blood</i> 2005	R-CHOP vs CHOP	223 vs 205	1.5 years	96% vs 90%	88% vs 70% (2-year DOR)	95% vs 90% (2-year OS)
Marcus et al, <i>J Clin Oncol.</i> 2008	R-CVP vs CVP	162 vs 159	4.5 years	81% vs 57%	38 months vs 14 months (median DOR)	83% vs 77% (4-year OS)

CVP, cyclophosphamide, vincristine, prednisone; DOR, duration of response; DFS, disease-free survival; EFS, event-free survival; R-CVP, rituximab, cyclophosphamide, vincristine, prednisone.

patients, BR therapy was found to be noninferior to the R-CHOP/R-CVP control arm for CR rate (30% vs 25%) and overall response rate (99% vs 94%). Time-to-event data were not reported. Side-effect profiles were distinct, with more GI toxicity and rash with BR and more neuropathy and alopecia with R-CHOP/R-CVP. Although, the BRIGHT data do not exactly replicate the StIL data for BR, they do suggest that BR remains a very reasonable alternative to R-CHOP or R-CVP in FL.

The question of whether to administer maintenance rituximab after frontline R-chemotherapy was addressed in the phase 3 PRIMA trial. The study evaluated the efficacy and safety profile of maintenance rituximab in newly diagnosed FL patients who responded to initial treatment with rituximab plus chemotherapy. Chemotherapy backbone was selected by treating center: R-CHOP (75%), R-CVP (22%), or R-FCM (3%). Patients were randomized to observation or to a single dose of rituximab every 2 months for 2 years. At a median follow-up of 36 months from randomization, the 2-year PFS in the maintenance rituximab arm was 75% versus 58% in the observation arm ($P < 0.0001$). The beneficial effect of maintenance rituximab was seen irrespective of the induction chemotherapy backbone and in both CR and partial remission (PR) patients. Grade 3–4 adverse events were slightly higher in the maintenance rituximab arm (24% vs 17%). No difference in OS was observed. Given the lack of OS benefit, the decision regarding the use of maintenance rituximab can be individualized. Rituximab administration does carry a low risk for neutropenia and low-grade infections, rarely, more serious toxicities, such as progressive multifocal leukoencephalopathy. As maintenance, rituximab generally is well tolerated and it has become a commonly utilized strategy in the United States.

More recently, the next-generation anti-CD20 monoclonal antibody obinutuzumab was compared with rituximab when combined with initial chemotherapy followed by maintenance in high-tumor-burden patients with follicular lymphoma. A total of 1,202 patients were randomized to obinutuzumab-chemo followed by obinutuzumab maintenance, vs rituximab-chemo followed by rituximab maintenance. Choice of chemotherapy backbone was at the discretion of participating centers and included bendamustine (57%), CHOP (32%), and CVP (10%). Dosing was different for the two antibodies, with obinutuzumab patients receiving more monoclonal antibody. Rituximab was administered at the standard dose of 375 mg/m^2 on day 1 of each chemoimmunotherapy cycle, while obinutuzumab was dosed at 1,000 mg on days 1, 8, and 15 during cycle 1, and then on day 1 of subsequent chemoimmunotherapy cycles. Maintenance was administered at the

same dose of the respective antibodies every 2 months for up to 2 years. The study showed no difference in overall or complete response rate between the two antibody strategies at the end of induction. During the maintenance period, however, a PFS benefit emerged in favor of obinutuzumab therapy with 3-year PFS of 80.0% vs 73.3%, and a hazard ratio of 0.66 (95% confidence interval, 0.51–0.85, $P = .0001$). There was no difference in OS, and toxicity was increased in the obinutuzumab arm with higher rates of neutropenia and infusion-related reactions. Based on these data, obinutuzumab-based chemoimmunotherapy plus maintenance is now an FDA approved initial treatment option for high-tumor-burden FL patients, but, in the absence of an OS benefit and with increased toxicity, rituximab-based therapy also continues to be an acceptable alternative. Notably, all patients in this trial received induction therapy followed by maintenance therapy, so, for patients planned for treatment with induction therapy alone without maintenance, rituximab-based treatment remains the most appropriate therapy.

Therapy for relapsed and refractory follicular lymphoma

Multiple options exist for the treatment of patients who have progressed after first-line therapy, and the decision of which therapy to use depends on a number of factors, including the prior treatment utilized, duration of prior response, patient age, comorbid illnesses, and goals of therapy. Options range from low-risk strategies, such as single-agent rituximab, to higher intensity strategies, such as autologous or allogeneic stem-cell transplantation, with many options in between. Population-based data and a report from the national LymphoCare study both show that patients who relapse within 2 years of initial chemoimmunotherapy have a significantly inferior overall survival compared to patients with longer initial remissions. Among the 80% of patients who enjoy an initial remission longer than 2 years, their predicted life expectancy is no different when compared to age-matched controls without lymphoma.

These high-risk patients with early progression of disease constitute an unmet medical need within relapsed FL and warrant evaluation in clinical trials of novel treatment approaches.

Bendamustine is approved in the United States for use in patients with rituximab-refractory indolent B-cell lymphoma. A pivotal trial in 100 patients reported an objective response rate (ORR) of 75% with a median PFS of 9.3 months. A subsequent randomized trial compared bendamustine alone to bendamustine combined with obinutuzumab, followed by obinutuzumab mainte-

nance, in rituximab-refractory FL. Patients treated with obinutuzumab-bendamustine demonstrated an improved PFS and OS compared to bendamustine alone, making this a preferred option in rituximab-refractory patients. An important caveat is that patients in this trial were bendamustine naïve, so this strategy has not proven beneficial in patients already treated with bendamustine therapy in the frontline setting.

Novel targeted therapies are playing an increasing role in the management of relapsed and refractory follicular lymphoma. The oral immunomodulating agent lenalidomide was evaluated as monotherapy or in combination with rituximab in a randomized trial for rituximab-sensitive FL, with lenalidomide -rituximab demonstrating an ORR and CRR of 76% and 39%, respectively, and a median time to progression of 2 years. Lenalidomide can now be considered an effective therapy for relapsed FL and is currently under evaluation as frontline therapy. Two targeted inhibitors of PI3K delta are also now available for patients with FL who have relapsed after at least two prior lines of therapy. The oral PI3K delta inhibitor idelalisib was evaluated in a phase 2 study of 125 patients with indolent NHL who were considered refractory to both rituximab and an alkylating agent. Idelalisib was administered at a dose of 150 mg BID until PD or patient withdrawal. The response rate was 57% with a median duration of 12.5 months. Grade 3 or higher toxicities included neutropenia (27%), transaminase elevations (13%), diarrhea (13%), and pneumonia (7%). Copanlisib, an intravenous inhibitor of PI3K delta and alpha, was also FDA-approved for this indication based on a phase 2 study in 142 patients with relapsed or refractory indolent lymphoma which had relapsed after at least 2 prior therapies. Copanlisib was administered intravenously on days 1, 8, and 15 of a 28-day cycle and continued until progression or intolerance. The ORR was 59% including 12% CRs and a median duration of response of 22.6 months. The most common grade 3–4 toxicities included hyperglycemia (41%), hypertension (24%), neutropenia (24%), and pneumonia (15%). More recently, the oral PI3K inhibitor duvelisib also demonstrated significant clinical activity in multiply relapsed FL with a similar safety profile to the other agents. All three PI3K inhibitors are FDA-approved for FL patients who have relapsed after at least 2 prior lines of therapy and represent effective treatment options in multiply relapsed/refractory disease, but their use in therapy requires counseling and monitoring for their unique toxicity profiles.

Radioimmunotherapy (RIT) is also an option for patients with indolent B-cell NHL if the bone marrow is minimally involved and the disease is not bulky. With Y⁹⁰

ibritumomab tiuxetan, response rates are ~70% and response duration is, on average, 11–15 months. Single-agent rituximab can be used in relapsed lymphoma, although now that most patients have received it with their primary therapy, and often as maintenance therapy, more and more patients are becoming rituximab-refractory. For patients who are still rituximab-sensitive, single-agent rituximab is an attractive option for elderly or unfit patients.

Stem-cell transplantation

HDC with autologous stem-cell transplantation (ASCT) and allogeneic stem-cell transplantation (allo SCT) are both useful strategies in the management of selected patients with FL, particularly for younger patients with high-risk features, such as a brief remission after initial therapy. A review of 904 patients in the International Bone Marrow Transplant Registry who underwent autologous or allogeneic transplantation for FL revealed that durable remissions could be induced with either technique. A lower 5-year recurrence rate with allogeneic transplantation was offset by a higher treatment-related mortality (TRM) compared with autologous transplantation, leading to similar 5-year survival rates of 51% to 62%. To reduce the TRM of allo SCT, a nonmyeloablative strategy is preferred in FL. Results utilizing a nonmyeloablative allogeneic SCT strategy vary widely in the literature. For example, a series of 62 patients treated at the Fred Hutchinson Cancer Research Center demonstrated a 3-year OS and PFS of 67% and 54%, respectively. Alternatively, a highly selected group ($n=47$) treated at the MD Anderson Cancer Center achieved an 11-year OS and PFS of 78% and 72%, respectively.

There is one small, randomized clinical trial (the CUP trial) examining ASCT versus standard therapy in patients with relapsed FL. The study, conducted in the pre-rituximab era, found improved PFS and a trend toward improved OS. An interesting long-term analysis of patients receiving myeloablative chemotherapy followed by ASCT comes from investigators at St. Bartholomew's Hospital (London) and the Dana-Farber Cancer Institute (Boston). A cohort of 121 patients, with a median follow-up of 13.5 years, was noted to have a plateau in the remission-duration curve beginning around year 8. Nearly half the patients were still in remission at 10 to 15 years, suggesting some patients may be cured. Results were substantially better for patients treated in second remission as opposed to later in the disease course, suggesting there may be an optimal window to consider ASCT in FL.

Patients who relapse within 2 years of their initial chemoimmunotherapy are at high risk of dying from FL with

a 5-year OS of approximately 50%. Retrospective analyses have been conducted to see if these high-risk patients might benefit preferentially from ASCT in the management of their relapsed disease. Data from the National Lympho-Care Study and Center for International Bone Marrow Transplant Research (CIBMTR) indeed showed no benefit in OS among all FL patients undergoing ASCT but did show an improved OS in the subgroup of patients with early progression of disease.

Marginal-zone lymphomas

The WHO classification separates the marginal-zone B-cell lymphomas (MZL) into extranodal MZL of MALT type, nodal MZL, and splenic MZL (SMZL). The morphology of these disorders is characterized by an infiltrate of centrocyte-like small cleaved cells, monocytoid B cells, or small lymphocytes; these disorders may exhibit an expanded marginal zone surrounding lymphoid follicles. The immunophenotype is characterized by expression of CD20 but lack of CD5 or CD10 expression (Table 23-2); this marker profile is useful in distinguishing MZL from SLL, MCL, and FL. A feature common to many cases of MZL is association with chronic antigenic stimulation by microbial pathogens or autoantigens as described above. Examples include gastric MALT (*H pylori*), cutaneous MALT (*B burgdorferi* or *afzelii*), ocular adnexal MALT (*C psittaci*), nodal MZL (hepatitis C), SMZL (hepatitis C), pulmonary or parotid MALT (Sjögren syndrome), and thyroid MALT (Hashimoto thyroiditis). There is significant geographic variation associated with certain microbial pathogens. For example, the prevalence of *C psittaci* in patients with ocular adnexal MALT appears to be 50% to 80% in Italy, Austria, Germany, and Korea, whereas this organism is observed infrequently in Japan, China, and the United States.

MALT lymphomas

Extranodal MZLs or MALT lymphomas constitute ~70% of all MZLs. They occur most commonly in mucosal sites, predominantly gastric or intestinal, as well as lung, salivary gland, ocular adnexa, skin, and thyroid, among others. These sites often are affected by chronic infection or inflammation in the setting of autoimmune disease, such as Sjögren syndrome or Hashimoto thyroiditis. The typical presentation of MALT lymphoma is an isolated mass in any of these extranodal sites or an ulcerative lesion in the stomach. Clinically, these lymphomas are typically indolent, with 10-year OS rates in excess of 90% in many series. MALT lymphomas can be characterized as gastric (30%-40%) or nongastric (60%-70%), and the approach to

disease management is site-specific. Approximately 90% of gastric MALT lymphomas are associated with *H pylori* infection. Newly diagnosed patients typically report dyspepsia, pain, reflux symptoms, or weight loss. Upper endoscopy can reveal erythema, erosions, ulcers, or masses. A consistent observation has been that 70% to 80% of gastric MALT lymphomas durably regress following effective *H pylori* antibiotic therapy. The most widely used antibiotic regimen is a combination of amoxicillin, omeprazole, and clarithromycin. Metronidazole is an effective alternative antibiotic in patients with a penicillin allergy. Lymphoma responses can be slow, taking as long as 6 months to 1 year. Repeat assessment of *H pylori*, by histologic examination or a urea breath test, is necessary to ensure that the bacteria have been eradicated. The strongest predictor for lymphoma nonresponse to antibiotic therapy is the presence of the t(11;18) translocation, which is present in 20% to 30% of cases. In the series reported by Nakamura et al, only 3 of 30 patients with t(11;18) experienced lymphoma regression following *H pylori* eradication therapy. In patients who do not respond to antibiotics, or in *H pylori*-negative cases, involved-field radiotherapy (IFRT) has been highly effective with DFS or PFS rates of >90% at 10 years. The prognosis for early-stage gastric MALT is excellent, with most series reporting 10-year OS rates in excess of 90%. For patients with advanced-stage disease, regimens similar to those used in FL, including rituximab alone or in combination, can be used. Transformation to DLBCL is possible, but a remarkable observation has been the regression of early-stage *H pylori*-positive gastric-diffuse large B-cell lymphomas with *H pylori*-eradication therapy. This observation was noted in DLBCL clearly arising from gastric MALT (transformation) and in de-novo DLBCL (no apparent underlying MALT).

Nongastric MALT lymphomas also have an indolent course, including the one-third of patients who present with stage 4 disease. OS at 10 years exceeds 90% in many series. The most common locations are the salivary glands (26%), ocular adnexa (17%), skin (12%), lung (8%), upper airways (7%), thyroid (6%), and intestinal tract (5%). Treatment approaches depend on both stage and site of primary involvement and may include surgery, radiation therapy, or chemotherapy. Radiation therapy produces excellent results in limited-stage disease. Many patients can be managed with surveillance alone if asymptomatic. Patients with advanced-stage disease typically can be managed using the same principles used for FL. Patients often have a low disease burden, and rituximab monotherapy may be highly effective. For high-tumor-burden patients or those progressing on rituximab alone, rituximab added

to chlorambucil was shown to improve EFS in an RCT compared to chlorambucil alone. Recurrences tend to occur in the same or other extranodal locations. For patients requiring chemoimmunotherapy, bendamustine has been employed with success, as with FL. Recently the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib was FDA-approved for relapsed/refractory marginal-zone lymphoma based on a 63-patient phase 2 trial for relapsed/refractory marginal-zone lymphoma of any subtype. The oral dose was 560 mg daily. Ibrutinib produced an ORR of 48% with a median PFS of 14.2 months, making this an appealing available option for patients with relapsed marginal zone lymphoma.

Nodal MZL

Nodal MZL also arises from marginal-zone B cells but presents with nodal involvement akin to FL. Whenever nodal MZL is diagnosed, a careful history review and a physical examination should be conducted to determine if a coexisting extranodal MALT lymphoma component exists, as concurrent disease may be present in up to one-third of cases. Nodal MZL more commonly presents at advanced-stage (Ann Arbor stage III-IV) than with MALT-type MZL. The t(11;18) karyotypic changes identified in MALT are absent in nodal MZL, and no specific or recurring karyotypic anomaly has been described. IgM monoclonal gammopathy can occur in ~10% of cases. HCV infection is reported in up to 25% of patients. Across reported series, the 5-year OS for nodal MZL is 60% to 70%; however, the EFS is only 30%, which likely reflects more commonly encountered advanced-stage disease. Management is similar to the approach recommended in FL, and ibrutinib is available as an option at relapse, as reviewed above.

In the updated WHO classification, a new category, pediatric nodal MZL, which has distinctive clinical and morphologic characteristics, was introduced. There is a male predominance (20:1), and patients usually present with localized asymptomatic adenopathy in the head and neck region. Morphologically, the infiltrate is similar to that seen in adults, except that progressively transformed germinal centers often are seen.

Splenic MZL

Splenic MZL (SMZL) presents at a median age of 68 years and is more common in females. Patients usually present with symptomatic splenomegaly, and involvement of the peripheral blood and bone marrow are common. Generalized lymphadenopathy is rare, but patients may have splenic hilar nodal or hepatic involvement. Patients may

have concomitant autoimmune cytopenias, which should be considered in patients with anemia or thrombocytopenia at diagnosis. Diagnosis usually is based on spleen histology following splenectomy or after bone-marrow examination. Clinically, SMZL can be confused with CLL, MCL, FL, HCL, or WM. Unlike CLL and MCL, SMZL is typically CD5-negative, and, unlike FL, it is CD10-negative. Unlike HCL, which is CD103-positive and replaces the splenic red pulp, SMZL is CD103-negative and replaces the splenic white pulp. WM may be distinguished from SMZL based on the presence of a *MYD88* mutation which does not occur in MZL. A prognostic model, using hemoglobin <12 g/dL, elevated LDH, and albumin <3.5 g/dL, has identified three distinctive risk groups (low, intermediate and high). OS at 5 years was 88%, 73%, and 50% for patients with 0, 1, and 2 or 3 risk factors, respectively, in the pre-rituximab era. All patients should be checked for underlying hepatitis C because antiviral therapy for hepatitis C often leads to regression of the SMZL and is the recommended initial treatment of choice in these patients. For non-hepatitis C patients, observation alone is the recommended initial approach for asymptomatic patients without bulky splenomegaly or significant cytopenias. For patients requiring therapy, splenectomy has long been considered the optimal first-line treatment. However, single-agent rituximab is also remarkably active, with an ORR approaching 100% in small series. In an observational retrospective study, rituximab produced more durable remissions than did splenectomy. For young patients, who are appropriate surgical candidates, splenectomy or rituximab monotherapy may be considered as initial therapy, whereas for elderly patients or patients otherwise unfit for surgery, rituximab monotherapy is preferred. Patients with subsequent relapses in need of therapy may be considered for splenectomy if not yet performed, retreatment with single-agent rituximab, or treatment with chemoimmunotherapy or ibrutinib.

Lymphoplasmacytic lymphoma and Waldenström macroglobulinemia

Lymphoplasmacytic lymphoma (LPL) is defined in the WHO classification as an indolent neoplasm of small B lymphocytes, plasmacytoid lymphocytes, and plasma cells. The lymphoma cells may express B-cell markers CD19 and CD20 and are CD5- and CD10-negative, much like the MZLs (Table 23-3). LPL with production of an IgM paraprotein produces the syndrome known as Waldenström macroglobulinemia, which is described further in Chapter 25.

Hairy cell leukemia

HCL is an indolent B-cell lymphoproliferative disorder accounting for only 2% of all leukemias; it is characterized pathologically by neoplastic lymphocytes with cytoplasmic “hairy” projections on the cell surface, a positive tartrate-resistant acid phosphatase stain, and an immunophenotype positive for surface immunoglobulin, CD19, CD20, CD22, CD11c, CD25, and CD103 (Table 23-2). Marrow biopsy demonstrates a mononuclear cell infiltrate with a “fried egg” appearance of a halo around the nuclei and increased reticulin and collagen fibrosis. Nearly 100% of cases harbor the *BRAF* V600E mutation, abnormally activating the *BRAF*-MEK-ERK pathway.

HCL is 4 times more common in men than in women and presents at a median age in the 50s with pancytopenia and splenomegaly. Most patients have an absolute monocytopenia, which may be a clue to the diagnosis. The bone marrow aspirate is often a dry tap due to increased marrow reticulin. Making the proper diagnosis is crucial because of HCL’s generally favorable prognosis, with a 10-year OS exceeding 90% and an excellent treatment response to nucleoside analogs. Most patients with HCL require therapy to correct cytopenias and associated complications, in addition to the presence of symptomatic splenomegaly. If a patient is asymptomatic and cytopenias are minimal, the patient may be observed initially. HCL is uniquely sensitivity to purine analogs. The nucleoside analogs cladribine or pentostatin are the treatments of choice in HCL in view of the high response rates and durable remissions achieved. Cladribine is used more commonly because of the short duration of therapy required; cladribine also is available as a subcutaneous injection. In one large series of 233 patients with long-term follow-up, the ORR and CR rates with either of these agents were 97% and 80%, respectively. The median recurrence-free survival was 16 years, and many of the relapses were observed 5 to 15 years after initial treatment, highlighting the unique natural history of this disease. It currently is recommended that assessment of response should be determined 4 to 6 months after the end of treatment; a second course can be given only if a PR is attained. Patients who relapse after frontline nucleoside analogue therapy are often retreated with a nucleoside analogue with similarly high response rates. Rituximab may also be administered for relapsed disease. For multiply relapsed patients, the anti-CD22 antibody drug conjugate moxetumomab pseudotox-tdfk is FDA approved for HCL relapsed after at least 2 prior therapies including a purine analog. Among 80 patients treated, the ORR was 75%, and the rate of durable CR (at least 180 days) was 30%. For the uncommon patients with relapsed HCL, who

are refractory to both nucleoside analogues and rituximab, *BRAF* inhibitors have also demonstrated high response rates as single agents and should be considered in these selected cases.

HCL-variant is a distinct disease categorized separately in the WHO classification, and, despite its name, it is considered to be unrelated to HCL. HCL-variant does not harbor the *BRAF*-V600E mutation. It differs from HCL in the lack of monocytopenia and by the presence of an elevated white blood cell count. The bone marrow is easier to aspirate because the reticulin fiber content is low. The immunophenotype of HCL-variant also differs in that the cells are CD25-negative. CD103 is expressed infrequently and CD11c is usually positive. Unlike HCL, HCL-variant responds poorly to purine analogs. Splenectomy can result in partial remissions, and some patients can respond well to rituximab.

Transformation to aggressive lymphoma in indolent lymphomas

Histologic transformation (HT) is the development of aggressive NHL in patients with an underlying indolent lymphoma. It most commonly occurs in FL but can occur in any of the indolent lymphomas. The British Columbia Cancer Agency reported on the incidence and outcome of 600 patients with FL who subsequently developed transformed lymphoma. Diagnoses were made clinically (sudden increase in LDH >2× the upper limit of normal, discordant nodal growth, or unusual extranodal sites of involvement) (37%) or pathologically (63%). In this series, the annual risk of transformation was 3% per year, with 10- and 15-year risks of 30% and 45%, respectively. Overall, the median post-transformation survival time was 1.7 years, with superior outcomes observed in limited-stage patients. Similar results were observed in a series from St. Bartholomew, where histologic transformation was observed in 28% of patients with FL by 10 years. A more recent analysis in the rituximab era, however, demonstrates a lower overall rate of HT in FL of 15% and with an improved outcome. FDG-PET imaging can be helpful in selecting a biopsy site when establishing HT, but bright FDG avidity alone does not establish a diagnosis of HT. Histologically, DLBCL is the most frequently observed subtype. One should assay for MYC and BCL-2 by FISH and by immunohistochemistry. The treatment is directed at the aggressive lymphoma and depends on a variety of factors, including age, comorbidities, and extent of prior treatment for FL. Patients with HT, who have never received R-CHOP, have a cure rate similar to de novo DLBCL, making R-CHOP the treatment of choice in most patients. Consideration for stem-cell transplantation consolidation is warranted in selected patients.

KEY POINTS

- Follicular NHL is the most common indolent NHL.
- Patients with asymptomatic, advanced-stage indolent NHL may be followed without specific therapy to assess the pace of disease, or single-agent rituximab may be used to delay the use of systemic chemotherapy.
- Anti-CD20 antibody therapy plus chemotherapy is recommended in patients with symptomatic or high-tumor-burden disease by the GELF criteria.
- Maintenance anti-CD20 antibody therapy improves PFS with no impact on OS.
- There are a multitude of therapeutic options for relapsed indolent lymphoma, including novel targeted agents and stem-cell transplantation.

DLBCL constitutes approximately 30% of all NHLs and can present with nodal or extranodal disease. Bone-marrow involvement with large-cell lymphoma occurs in fewer than 10% of cases. Another 10% to 20% of patients have discordant marrow involvement with a low-grade B-cell lymphoma, despite a nodal biopsy consistent with DLBCL.

In addition to the B-cell markers CD20 and CD19, the neoplastic cells may also express CD10 (30% to 60%), BCL6 (60% to 90%), and IRF4/MUM1 (35% to 65%). Rare cases may express CD5 (10%) and must be distinguished from the blastoid variant of MCL, which is cyclin-D1-positive. As described, two molecularly distinct subtypes of DLBCL NOS are recognized: GCB, which has a gene-expression profile similar to germinal-center B cells (CD10⁺ and BCL6⁺); and activated B-cell (ABC), which has a profile similar to activated peripheral B cells (IRF4/MUM⁺) with a prominent *NFKB* gene signature.

Aggressive B-cell lymphomas

DLBCL is the prototypical aggressive lymphoma, with other histologies including MCL, BL, peripheral T-cell lymphomas, anaplastic large-cell lymphoma, and others (Table 23-3). These neoplasms are typically characterized by rapidly progressing nodal or extranodal disease and, although often potentially curable, are associated with relatively short survival in the absence of successful therapy. This chapter focuses on the mature B- and T-/NK-cell neoplasms.

CLINICAL CASE

A 52-year-old man is diagnosed with stage IVB DLBCL. On PET-CT imaging, the largest nodal mass was 6 cm in the retroperitoneal region, and there was lymphoma involvement of liver and bone. Laboratory studies show a normal complete blood count (CBC) and normal chemistries, aside from an LDH elevated 1.5 times normal. His Eastern Cooperative Oncology Group performance status (ECOG PS) is 1. Immunophenotypic stains of the lymphoma cells revealed expression of CD19, CD20, κ light chains, BCL2, MYC, and MUM1/IRF4. Lymphoma cells were negative for CD10 and BCL6 expression.

Clinical prognostic factors in DLBCL

Approximately two-thirds of patients diagnosed with DLBCL can be cured with rituximab-based chemotherapy; however, low- and high-risk groups can further be defined by clinical and biological factors. Although the IPI is robust and relevant in the modern rituximab treatment era, it does not capture all prognostic information. The patient described earlier has an IPI score of 3 (advanced-stage, multiple sites of extranodal involvement, elevated LDH), placing him in a high-intermediate-risk group with an expected 5-year probability of survival with R-CHOP of 50% to 60%.

Biological prognostic factors in DLBCL

Although the IPI is easy to apply and remains valid in the current treatment era, it fails to capture underlying biological heterogeneity. As described above, DLBCL can be divided molecularly by gene-expression profiling (GEP) into the germinal-center B-cell (GCB) and activated B-cell (ABC) subtypes, which also have a signature distinct from PMBCL. ABC DLBCL has an inferior prognosis, independent of the IPI. The use of GEP has had limited clinical utility due to long turnaround time, the need to use fresh frozen tissue, technical complexity, and lack of routine availability in the clinic.

Immunohistochemical (IHC) algorithms have been used in an attempt to capture the cell-of-origin (COO) phenotype using a methodology that can be applied routinely in clinical practice. Hans et al first reported an IHC algorithm to distinguish the GCB versus non-GCB subgroups using CD10, BCL6, and IRF4/MUM1. Using the cDNA microarray as the gold standard, the sensitivity of the IHC COO subgrouping was 71% for the GCB group and 88% for the non-GCB group. Other algorithms have

Diffuse large B-cell lymphoma

DLBCL is composed of large B cells with a diffuse growth pattern. The WHO classification recognizes several sub-categories of DLBCL, including molecular subtypes (GCB and ABC; see later sections); pathologic subtypes, including T-cell/histiocyte-rich large B-cell lymphoma; and defined disease entities, including primary mediastinal large B-cell lymphoma (PMBCL) and primary DLBCL of the CNS.

been proposed that also have a lower sensitivity than gene-expression profiling. These results, however, have been inconsistent as to whether the COO distinction by IHC can be applied to rituximab-treated patients. One study found that none of the applied five different IHC algorithms could distinguish COO subgroups with prognostic significance. In contrast, another study found that the Tally algorithm, which uses CD10, GCET, IRF4/MUM1, and FOXP1, showed the best concordance with microarray data and maintained prognostic significance. Given these inconsistencies and the lack of data suggesting that alternate therapies may affect outcome, the COO information, whether by molecular profiling or immunohistochemistry, should not be used to direct treatment decisions outside of clinical trials.

Recent technological advances in GEP, allows real-time COO determination from formalin-fixed paraffin-embedded tissue (FFPET). The Lymphoma/Leukemia Molecular Profiling Project developed the Lymph2Cx assay, a parsimonious digital gene-expression (NanoString)-based test for COO assignment in FFPET. A 20-gene assay was trained using 51 FFPET biopsies, and the locked assay was subsequently validated using an independent cohort of 68 FFPET biopsies. Comparisons were made with COO assignment using the original COO model on matched frozen tissue. The assay was highly accurate; only 1 case with definitive COO was incorrectly assigned with >95% concordance of COO assignment between two independent laboratories. The test turnaround time is several days, making Lymph2Cx attractive for implementation in clinical trials and practice. However, until gene-expression analysis becomes clinically available, the 2016 WHO classification includes subclassification of DLBCL NOS as GCB or non-GCB based on IHC algorithms.

MYC is translocated in ~5% to 10% of DLBCLs, and early studies have suggested that *MYC* is associated with an aggressive course in the pre- and post-rituximab treatment eras. In some cases, there is also a t(14;18) involving *BCL2*, or a *BCL6* translocation involving chromosome 3, in which case the disease has been dubbed double-hit lymphoma (DHL) or triple-hit lymphoma (THL), if all three translocations are present. DHL/THL can occur as a high-grade transformation from an underlying FL or as a de-novo disease. The combination of *MYC* driving cellular proliferation and *BCL2* preventing apoptosis has proven to be an extremely high-risk biologic subset of aggressive lymphomas with low cure rates using traditional R-CHOP. In the previous 2008 WHO classification scheme, DHLs were incorporated within the classification of DLBCL or, more commonly, of B-cell lymphoma, unclassifiable with features intermediate between BL and diffuse large B-cell

lymphoma. In the 2016 WHO, DHL is now a distinct molecularly defined aggressive lymphoma called “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements” and the previous classification of “B-cell lymphoma, unclassifiable with features intermediate between BL and diffuse large B-cell lymphoma” has been eliminated. With the recent availability of a *MYC* antibody for IHC analysis, two large-scale studies have evaluated the prognostic importance of *MYC*- and *BCL2*-protein expression (double expressers) in DLBCL patients treated with R-CHOP chemotherapy. *MYC* protein expression was found in approximately one-third of cases, a higher incidence than that captured by fluorescence in situ hybridization (FISH) analysis (11%) or high *MYC* mRNA expression, suggesting that multiple roads of *MYC*-deregulation exist. Importantly, the double expressers, which account for 20% to 25% of newly diagnosed DLBCLs, have an inferior prognosis relative to other DLBCLs, though not as poor as for patients with DHL. Novel treatment approaches for these high-risk patients are needed.

Treatment of newly diagnosed DLBCL

Advanced-stage DLBCL

The backbone of treatment of all subtypes of DLBCL is anthracycline-based treatment with R-CHOP chemotherapy. With this approach, approximately two-thirds of patients are cured.

Rituximab has several mechanisms of action, including the ability to sensitize otherwise-resistant lymphoma cells to chemotherapy agents in vitro, perhaps, in part, via downregulation of the *BCL-2* protein. GELA published a landmark phase 3 clinical trial in which 399 patients 60 to 80 years of age, with previously untreated advanced-stage CD20+-DLBCL, were randomized to receive CHOP for 8 cycles or R-CHOP on a standard 21-day schedule. R-CHOP demonstrated an improvement over CHOP for all endpoints, including CR rate, EFS, and OS. With longer follow-up, the results held, and R-CHOP quickly became the standard of care for advanced-stage DLBCL around the world (Table 23-12). More recently, the median 10-year-outcome of patients in this study demonstrated a 10-year PFS for R-CHOP-treated patients of 35% (vs 20% for CHOP alone) and a 10-year OS of 43.5% (vs 27.6% for CHOP alone) (Table 23-12). A similar phase 3 study was carried out by the US ECOG intergroup (E4494) study comparing 6 to 8 cycles of CHOP versus R-CHOP in elderly patients with aggressive lymphoma, which included a second randomization in CR patients comparing observation and rituximab maintenance therapy every 6 months for 2 years. Unlike the GELA study, there was no response-rate or OS difference detected, although

Table 23-12 Key trials of diffuse large B-cell lymphoma using rituximab-containing regimens

Author (trial/phase)	N	Treatment	Patient selection	PFS/EFS	OS
Coiffier et al, <i>N Engl J Med.</i> 2002 (GELA/III)	202	R-CHOP×8 vs	Age 60–80 y Stage II-IV	57% vs 38% (2 y)	70% vs 57% (2 y)
	197	CHOP×8			
Pfreundschuh et al, <i>Lancet Oncol.</i> 2006 (MInT/III)	413	R-CHOP-like [‡] ×6 vs	Age 18–60 y aaIPI 0 or 1 Stage I (+bulk or II-IV)	74% vs 56% (6 y)	90% vs 80% (6 y)
	410	CHOP like [‡] ×6			
Pfreundschuh et al, <i>Lancet Oncol.</i> 2008 (RiCOVER-60/III) [†]	306	R-CHOP-14×6	Age 61–80 y Stage I-IV	66.5% (3 y)	78% (3 y)
	304	R-CHOP-14×8		63% (3 y)	72.5% (3 y)
	209	CHOP-14×6		47% (3 y)	68% (3 y)
	219	CHOP-14×8		53% (3 y)	66% (3 y)
Cunningham et al, <i>Lancet</i> 2013 (NCRI/III)	540	R-CHOP-21×8	Age 61–80 y	81% vs 83% (2 y)*	81% vs 83% (2 y)*
	540	R-CHOP-14×6+ G-CSF			
Delarue et al, <i>Lancet Oncol.</i> 2013 (LNH03-6B/III)	296	R-CHOP-21×8	Age 60–80 y aaIPI >1	60% vs 56% (3 y)*	72% vs 69% (3 y)*
	304	R-CHOP-14×6			
Recher et al, <i>Lancet</i> 2011 (LNH03-2B/III)	196	R-ACVBP	Age 18–59 y aaIPI 1	87% vs 73% (3 y)	92% vs 89% (3 y)
	183	R-CHOP			

Survival estimates shown for rituximab-containing regimens only and are rounded off where applicable to the nearest whole number.

EFS, event-free survival; G-CSF, granulocyte colony-stimulating factor; GELA, Groupe d'Etude des Lymphomes de l'Adulte; MInT, MabThera International Study Group; NCRI, British National Cancer Research Institute Study; R, rituximab; RiCOVER-60, Rituximab with CHOP Over Age 60 Years.

*87% DLBCL; CHOP-like = CHOP-21 or CHOEP-21 in 92%; radiotherapy given to sites of bulk, extranodal disease (physician's discretion).

[†]80% DLBCL.

*P value not significant (all other P values for comparisons are significant).

there was a benefit in TTF for the R-CHOP arm. The analysis was confounded to some extent by the secondary randomization to maintenance vs no-maintenance rituximab. Maintenance therapy was beneficial for the TTF only in the CHOP-induction subset. As such, interpretation of these results supports the use of R-CHOP induction without subsequent maintenance rituximab therapy.

Two other randomized controlled studies have been published supporting the benefit of the addition of rituximab to anthracycline-based chemotherapy in DLBCL. The MabThera International Study Group (MInT) study included young (<60 years), low-risk (aaIPI 0 or 1) patients with DLBCL (including PMBCL) who primarily received CHOP or CHOP plus etoposide (CHOEP) with or without rituximab. The rituximab-containing regimens demonstrated an improvement in EFS and OS (Table 23-12). The RItuximab with CHOP OVER age 60 Years (RICOVER-60) trial by the same group evaluated CHOP-14 for 6 or 8 cycles, with or without rituximab in elderly patients and also demonstrated a significant improvement in all endpoints with the rituximab combinations. Of note, the latter study also established that 6 cycles of R-CHOP-14 was associated with the best outcome.

Two randomized studies (GELA LNH-03-6B and the British National Cancer Research Institute [NCRI]) compared R-CHOP-21 (ie, every 21 days) with R-CHOP-14 (every 14 days), and there was no improvement of FFS or OS using the shortened cycle interval, thus confirming that R-CHOP-21 remains the standard (Table 23-12). Based upon the observation that elderly females fare better with R-CHOP than do elderly males and that elderly males clear rituximab more rapidly, dose-dense rituximab regimens are being tested in elderly males. A trial, where elderly males were treated with higher dose of rituximab given at 500 mg/m² while females received standard dose of 375 mg, showed that outcomes for male patients treated with higher-dose rituximab was equivalent to outcomes of historically treated females. Several recent randomized trials have sought to improve upon R-CHOP results in DLBCL. Explored strategies compared to standard R-CHOP have included substituting the next generation anti-CD20 monoclonal antibody obinutuzumab for rituximab, addition of the proteasome inhibitor bortezomib, maintenance everolimus, consolidation with HDC and ASCT, and infusional therapy with dose-adjusted EP-OCH-R. All randomized trials showed no improvement

in survival over standard R-CHOP. Based on these data, administration of R-CHOP every 21 days for 6 cycles remains the standard of care for advanced-stage DLBCL.

Treatment of limited-stage DLBCL

Approximately 45% of cases of DLBCL are limited-stage, Ann Arbor stages I-II. A large randomized Southwest Oncology Group (SWOG) trial (SWOG-8736) in the pre-rituximab era established that CMT, including chemotherapy followed by radiation, was superior to CHOP alone for localized [stage I(E), nonbulky stage II(E)] aggressive lymphoma. In this study, the 5-year PFS (77% vs 65%, $P=0.03$) and OS (82% vs 72%, $P=0.02$) for three cycles of CHOP followed by IFRT was superior to that of 8 cycles of CHOP alone. An update of the study with longer follow-up, however, showed that the treatment advantage for the CMT was not sustained; there was an identical 10-year PFS of 55% in both treatment arms.

The benefit of rituximab has not been specifically analyzed in a randomized controlled trial in localized DLBCL. The majority of patients in the MInT study had limited-stage disease by nature of the inclusion criteria, and that study confirmed the benefit of rituximab in this population. The SWOG completed a phase 2 study evaluating 3 cycles of R-CHOP, with 4 doses of rituximab, followed by IFRT (40–46 Gy, if CR, and 50–55 Gy, if PR) in patients with localized aggressive B-cell lymphoma, most of whom had DLBCL. Patients had to have at least one risk factor by the stage-modified IPI and had a 10-year PFS and OS of 58% and 67%, respectively.

With potential acute and more concerning long-term side effects of radiotherapy, determining whether a subgroup of patients with limited-stage DLBCL can be selected to receive chemotherapy alone is an important issue. A French study in limited-stage nonbulky (<7 cm) DLBCL randomized patients to 4–6 cycles of R-CHOP followed by 40 Gy XRT or to 4–6 cycles of R-CHOP alone. Patients with an IPI score of 0 received 4 cycles, while patients with IPI scores of ≥ 1 received 6 cycles. Only patients in a CR by PET-CT were randomized between chemotherapy alone or CMT, while all PR patients received CMT. Eighty-eight percent of patients achieved a CR and were randomized, with no difference in 5-year EFS or OS between the treatment arms. These data validate chemoimmunotherapy alone as an appropriate treatment plan for nonbulky limited-stage DLBCL patients who achieve a CR to R-CHOP.

CR patients received 4 to 6 cycles of R-CHOP followed by 40-Gy RT. Patients in CR by PET imaging after 4 cycles (84%) did not receive cycles 5 and 6 of R-CHOP.

The patients assigned to no RT had EFS and OS that were not different compared to patients receiving RT, suggesting RT may be unnecessary in selected patients responding well to chemoimmunotherapy alone.

Primary testicular DLBCL represents a unique subset of DLBCL, most commonly presenting at limited-stage. These patients have a propensity for late relapse, as well as a high risk of CNS recurrence (parenchymal > leptomeningeal) and recurrence within the contralateral testis. As such, patients with primary testicular DLBCL are typically treated with 6 cycles of R-CHOP, including CNS prophylaxis, followed by prophylactic scrotal radiation to the contralateral testis.

Novel strategies to improve cure rates in DLBCL

Although the outcome of DLBCL has improved with R-CHOP chemotherapy, ~43% of patients still relapse after primary therapy, and most relapsing patients will not be cured of their disease. As noted earlier, multiple randomized trials have failed to identify therapy superior to R-CHOP. Ongoing trials are now seeking to incorporate novel target agents with a biologic rationale in discrete DLBCL subsets. Both lenalidomide and ibrutinib may be selectively beneficial in ABC-DLBCL, with each showing single-agent activity in relapsed ABC-DLBCL compared to GCB. Randomized trials are currently evaluating each of these agents in combination with R-CHOP compared to R-CHOP alone, specifically in ABC/non-GCB DLBCL. Results of these trials are eagerly anticipated and could change the standard of care in a biologically defined subset of DLBCL patients.

Management of relapsed and refractory DLBCL

Repeating a biopsy at the time of suspected recurrence is recommended given the implications of recurrent DLBCL and possibility of relapse with a different histology. Following confirmation of recurrence, patients should undergo full restaging investigations. If the patient does not have significant comorbidities and is younger than 70 years of age (younger than 80 in some centers), second-line (salvage) combination chemotherapy, such as R-ICE (rituximab, ifosfamide, carboplatin, etoposide), R-DHAP (rituximab, dexamethasone, Ara-C, cisplatin), or R-GDP (rituximab, gemcitabine, dexamethasone, cisplatin) should be given followed by HDC/ASCT, if chemotherapy-sensitive disease is demonstrated. The evidence supporting the use of HDC/ASCT in relapsed DLBCL is based on the historic Parma study (named after the city of Parma, Italy where the study group who conducted the trial first met). Patients, who relapsed with aggressive lymphoma (excluding CNS or bone

marrow involvement) following an initial CR to primary therapy, received 2 cycles of DHAP chemotherapy. If chemosensitivity (ie, a PR or CR to salvage chemotherapy) was demonstrated, patients were then randomized to receive further chemotherapy with DHAP or with HDC with BEAC (carmustine, etoposide, cytarabine, and cyclophosphamide) and ASCT. Patients in the transplantation arm had an improvement in both the 5-year EFS (46% vs 12%, $P=.001$) and OS (53% vs 32%, $P=.038$). Randomized trials in the modern era, however, have demonstrated disappointing success rates with this approach in patients who relapse or are refractory to R-CHOP, with fewer than 30% of patients remaining progression-free at 2 years.

The optimal salvage therapy recently has been investigated in 3 phase 3 randomized controlled trials. The Collaborative Trial in Relapsed Aggressive Lymphoma (CORAL) study randomized patients with relapsed DLBCL (or those who had not achieved a CR) to receive rituximab plus ifosfamide, carboplatin, and etoposide (R-ICE) or rituximab plus dexamethasone, high dose ara-C, and cisplatin (R-DHAP) for 3 cycles followed by HDC with carmustine (BCNU), etoposide, cytarabine and melphalan [BEAM]/ASCT if a response was demonstrated. There was also a second randomization following transplantation to rituximab or to observation to evaluate the role of maintenance therapy. At diagnosis, 62% of the patients had been treated with a CHOP-like regimen with rituximab. The ORR was similar between R-DHAP and R-ICE (63% vs 63.5%), and there was no difference in EFS or OS, and maintenance rituximab did not affect outcome. Patients who previously had received rituximab with their primary therapy had an inferior response rate (51% vs 83%, $P<.001$) and an inferior 3-year EFS (21% vs 47%), suggesting that these patients represent a very chemoresistant group. Additional poor prognostic factors that emerged from this study were early relapse <1 year and an aaIPI of 2 or 3. Interestingly, a subsequent correlative study suggested that patients with GCB DLBCL had an improved outcome to R-DHAP compared with R-ICE (3-year PFS 52% vs 32%, $P=.018$), which was even more striking if cases were defined by gene-expression profiling (GEP) (3-year PFS 100 % vs 27%), but the numbers were small. A second phase 3 trial was conducted by the NCIC (National Cancer Institute of Canada) comparing R-DHAP to the outpatient salvage regimen R-GDP (rituximab, gemcitabine, dexamethasone, cisplatin) in aggressive lymphomas using a noninferiority design. The ORR, EFS, and OS were similar between the treatment arms, but the R-GDP arm was associated with less grade 3 or 4 toxicity ($P=.0003$), including febrile neutropenia

(9% vs 23%, $P<.0001$); patients had superior QOL scores. Finally, a third randomized trial evaluated ofatumumab-DHAP vs R-DHAP as salvage therapy prior to ASCT in relapsed DLBCL and found no difference between the arms. The complete response rates to salvage therapy were low in both arms, and only 25% of patients remained progression-free at 2 years, highlighting treatment of relapsed DLBCL as a largely unmet medical need in the modern era. The primary predictor of success was achieving a CR by PET scan prior to ASCT.

Management of non-transplant-eligible patients with relapsed or refractory DLBCL, including novel therapies

Many patients relapse after HDC/ASCT or are not eligible for curative-intent treatment with salvage chemotherapy and HDC/ASCT due to advanced age or comorbidities. The goal of treatment in this setting is typically palliative; therefore lower intensity regimens are typically employed which may offer short-term disease control with modest treatment-associated toxicity. Commonly used regimens in this context include gemcitabine-based regimens, such as R-GemOx (rituximab, gemcitabine, oxaliplatin), or rituximab-bendamustine. Certain therapies may also be appealing in selected subsets of relapsed/refractory DLBCL. For tumors expressing CD30, the anti-CD30 antibody drug-conjugate brentuximab vedotin produces an overall response rate of 44% with a median duration of response of approximately 6 months and should be considered as an option in relapsed/refractory CD30⁺ DLBCL. Lenalidomide monotherapy produces responses in approximately one-quarter of relapsed DLBCL patients, but the response rate and durability represent the subset of patients with non-GCB DLBCL for whom this therapy should be considered. Similarly, the BTK inhibitor ibrutinib produces selectively higher responses in the ABC subset of DLBCL in whom the ORR was 37%. Interestingly, the pattern of mutations within the ABC DLBCL may help predict patients likelier to respond to ibrutinib. Patients harboring mutations of both *CD79B* and *MYD88* appear to have the highest likelihood of response, while *CARD11* and *TNFAIP3* mutations appear unlikely to respond.

Most recently, genetically modified autologous chimeric-antigen-receptor (CAR) T cells targeting CD19 have emerged as highly active agents in the management of chemotherapy-refractory DLBCL. The anti-CD19 CAR T-cell product axicabtagene ciloleucel (axi-cel) was evaluated in a phase 2 trial of 111 patients with chemotherapy-refractory DLBC, PMBCL, or transformed FL. Refractoriness to chemotherapy was defined as lack of response

to prior therapy or relapse within 1 year of HDC/ASCT. The median number of prior therapies was 3, and 21% had relapsed after ASCT. Among 111 enrolled patients, 101 patients were treated with axi-cel, while the remaining 10 subjects did not receive their infusion due to adverse events (4), lack of measurable disease (2), death from disease progression (1), and manufacturing failure (1). The overall response rate for treated patients was a remarkable 82%, with a complete response rate of 54%. At 1 year of follow-up, 42% of subjects remained in remission, demonstrating encouraging durability in a significant proportion of these high-risk patients. Toxicities from CAR T cells include cytopenias resulting from the lymphodepleting fludarabine and cyclophosphamide which precedes the CAR T-cell infusion, as well as toxicities related to cytokine release in the setting of in-vivo CAR T-cell expansion. Cytokine release syndrome (CRS) was observed in 93% of patients treated with axi-cel and was most commonly characterized by fever, hypoxia, and hypotension. CRS was severe (grades 3–4) in 13% of patients, and was almost entirely reversible, although there were 2 deaths. The syndrome is largely driven by release of IL-6, and treatment with the IL-6 receptor antagonist tocilizumab does help to rapidly reverse the syndrome in most patients without impairing efficacy of the treatment. The other common toxicity was a neurologic event, which occurred in 64% of patients (28% severe) and was most commonly encephalopathy, aphasia, or somnolence. As with CRS, most cases are entirely reversible, with steroids appearing to be the most effective therapy in severe cases. Based on these data, axi-cel was FDA-approved for DLBCL, PMBCL and transformed FL patients who had received at least 2 prior lines of therapy and is now the most effective therapy available for chemotherapy-refractory DLBCL. Given the complexity and toxicity profile of this therapy, it must be administered only at centers experienced in its use. Tisagenlecleucel is another recently FDA-approved anti-CD19 CAR T-cell for multiply relapsed or refractory DLBCL and transformed FL, with other products in development and likely to join the treatment armamentarium.

Special situations: management of specific clinicopathologic entities of DLBCL

Primary mediastinal (thymic) large B-cell lymphoma

PMBCL was recognized as a specific entity in the WHO classification based on unique clinicopathologic presentation. Unlike typical cases of DLBCL, PMBCL occurs at a median age of 35 years and is slightly more common in women than in men. Most patients present with a bulky anterior mediastinal mass that can invade the lung and

chest wall and occasionally can cause superior vena cava syndrome. Distant spread is uncommon at diagnosis, occurring in about one-quarter of patients. At relapse, involvement of visceral extranodal sites, including the kidneys, adrenals, ovaries, liver, and CNS, can occur.

Histologically, sclerosis is typically present, and phenotypically, the cells lack surface immunoglobulin expression but express B-cell markers, such as CD19 and CD20. CD30 expression is present in 80% of cases; however, it is usually weak and heterogeneous. Interestingly, gene-expression analysis has shown that PMBCL is molecularly distinct from typical DLBCL and shares many components of the molecular signature with cHL. It had long been speculated that there may be a pathogenic overlap between the nodular-sclerosis subtype of cHL based on shared clinical features, including a young age of onset and mediastinal predominance, as well as pathologic features, including predominant fibrosis and tumor cells that are CD30⁺. In addition, composite and sequential lymphomas have been reported, and a gray zone lymphoma (GZL) with overlapping features of both malignancies is now defined in the WHO classification (see the section “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL”), further highlighting the biological continuum between these diseases.

A novel recurrent translocation involving *CIITA* (MHC class II transactivator), found to be recurrent in PMBCL and occurring in 38% of patients, is also found in 15% of cHL (Table 23-2). Cases with these chromosomal breaks have an inferior disease-specific survival. Prior studies also found reduced expression of MHC class II genes, which also is linked to an inferior outcome. Additionally, PMBCL often has 9p24.1 amplifications that results in increased expression of PD-1 ligand, which is a rational therapeutic target (discussed below).

The outcome of patients with PMBCL is generally favorable, with a 5-year PFS of 70% when patients are treated with R-CHOP, though approximately 20% of patients have primary induction failure which can be very difficult to salvage. Given the typical bulky localized presentation, the majority of patients have historically also received consolidative radiation therapy, which exposes this population of predominantly young women to late radiation risks including breast cancer and heart and lung disease. The significant rate of primary refractory disease with R-CHOP and the need for radiation therapy in the majority of patients prompted evaluation of dose-adjusted etoposide + prednisone + vincristine + cyclophosphamide + doxorubicin + rituximab (DA-EPOCH-R) without radiation in a phase 2 study at the National Cancer Institute. Fifty-one patients, median age, 30 years, were treated. Fifty-nine

percent of patients were female, 65% had bulky disease ≥10cm, and 29% had stage IV disease. At a median follow-up of 5 years, 93% of patients were event-free, and the OS was 97%. These data have resulted in widespread adoption of DA-EPOCH-R without radiation therapy as the up-front treatment of choice for most patients with PMBCL.

Relapsed PMBCL is treated similarly to other relapsed DLBCLs, with second-line chemoimmunotherapy and HDC/ASCT being the treatment of choice for patients with chemosensitive disease. Unfortunately, PMBCL is often highly chemoresistant at the time of progression and has been historically very difficult to salvage with conventional therapy. For patients relapsing after ASCT, or not eligible for ASCT due to chemorefractory disease, the PD-1 inhibitor pembrolizumab has shown evidence of efficacy as has anti-CD19 CAR T-cell therapy, both of which are now available for chemotherapy-refractory PMBCL.

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL

Introduced in the WHO 2008 classification, this diagnosis was defined by overlapping clinical, morphological, or immunophenotypic features between cHL and DLBCL, particularly PMBCL. These cases of so-called GZL usually occur in young men between 20 and 40 years old who present with an anterior mediastinal mass and who may have supraclavicular lymph node involvement. A broad spectrum of cytological appearances can occur within the same tumor. The immunophenotype often is transitional between PMBCL and cHL (see Chapter 22) with the tumor cells CD45⁺, CD20⁺, CD30⁺, and CD15⁺. Cases of morphologically nodular sclerosis cHL with strong and uniform expression of CD20 and CD15 would favor a diagnosis of GZL. In contrast, cases resembling PMBCL but that are CD20⁻ and CD15⁺ or EBV⁺, also would support a diagnosis of GZL. Clinical outcomes appear inferior in GZL compared to PMBCL or HL, but higher remission rates have been observed with DLBCL-type regimens, such as R-CHOP or DA-EPOCH-R rather than Hodgkin lymphoma therapy. Given the increased risk of chemoresistance in this subset, consolidative radiation therapy should be considered in patients with localized disease.

T-cell/histiocyte-rich DLBCL

T-cell/histiocyte-rich DLBCL is an uncommon variant of DLBCL, which usually presents at advanced stage with frequent involvement of liver, spleen, and bone marrow. Typically, the neoplastic cells comprise <10% of cellular population and are outnumbered by a background of abundant T-cells and histiocytes. Histologically, it can resemble nodular lymphocyte predominant HL (NLPHL)

or can be transformed from a prior diagnosis of NLPHL. Treatment with R-CHOP leads to results similar to those seen in DLBCL NOS and remains the standard of care.

High-grade B-cell lymphoma with MYC and *BCL2* and/or *BCL6* rearrangements (double-hit lymphoma)

Five to 10% of DLBCL patients have DHL, defined as the presence of *MYC* and *BCL2* or *BCL6* translocations (detected by FISH or karyotype). These cases have mutational features, and frequently morphologic features, intermediate between DLBCL and BL and have been reclassified in the 2017 WHO classification as high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements. These high-risk patients have lower OS when treated with R-CHOP; therefore, R-CHOP is considered an inadequate therapy for the majority of patients with DHL, who have a median OS of approximately 2 years.

The majority of patients present with poor prognostic features, including advanced age, elevated LDH, and an advanced stage, often with extranodal involvement, including CNS. Patients may present with circulating leukemic-phase disease, which is extremely uncommon in typical cases of DLBCL. Due to inadequacy of R-CHOP therapy, various intensified chemoimmunotherapy strategies have been used, largely based on experience in BL; however, advanced age of most patients and often poor performance status limits the use of highly intensive chemotherapy. Due to rarity of DHL, data largely come from retrospective reviews, making comparison between regimens difficult. The intensified upfront induction regimens including R-HyperCVAD/MA and R-CODOXM/IVAC appear to compare favorably with historical controls treated with R-CHOP; however, one must bear in mind that patients who are candidates for such intensive therapy are frequently younger and have better PS; therefore, results may not be generalizable to a majority of patients with DHL. DA-EPOCH-R therapy does appear to perform better than R-CHOP in retrospective analyses and can be tolerated in older adults, leading to wide employment of this regimen for this disease. Given the high risk of CNS dissemination, prophylactic therapy for the CNS is recommended. Whether consolidative stem-cell transplantation offers additional benefit remains uncertain, but thus far retrospective analyses have not identified a clear benefit for transplantation in first remission for DHL. Novel agents for this disease are under investigation and are clearly needed. Encouragingly, patients with chemotherapy-refractory DHL have been shown to have responses to anti-CD19 CAR T-cell therapy analogous to patients with DLBCL NOS, and so should be considered for this treatment.

KEY POINTS

- Diffuse large B-cell lymphoma (DLBCL) is the most common histologic subtype of NHL.
- The IPI and cell-of-origin phenotype remain prognostic in the rituximab treatment era in DLBCL. Studies are ongoing to determine whether patients classified as high risk by the IPI or ABC phenotype should be treated with a therapy other than R-CHOP.
- Treatment with R-CHOP-21 (ie, repeated every 21 days) for 6 cycles is a standard of care in advanced disease; the role of consolidative radiation in advanced disease is not well defined.
- In limited-stage disease, abbreviated chemotherapy with 3–4 cycles of R-CHOP plus involved-field radiotherapy (IFRT) can be used. R-CHOP alone is an option for patients with nonbulky disease who achieve a CR on their PET-CT.
- Presence of relapsed disease should be documented by biopsy whenever possible.
- Transplantation-eligible patients with relapsed DLBCL are usually treated with salvage chemotherapy (RDHAP, RICE, and RGDP appear to have similar efficacy) followed by high-dose chemotherapy and stem-cell transplantation.
- Anti-CD19 CAR T-cell therapy can induce durable remissions in a significant proportion of chemotherapy-refractory DLBCL, PMBCL, and transformed FL.
- PMBCL patients should preferentially be treated with DA-EPOCH-R without RT, though there are no randomized studies in this disease.
- High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements (DHL) represents a particularly poor prognostic category when treated with R-CHOP; the disease is usually treated with more intensive regimens.

Primary CNS lymphoma

Primary CNS lymphoma (PCNSL) can occur in the brain parenchyma, spinal cord, eye (ocular) (Figure 23-3), cranial nerves, or meninges. Of note, although 95% of cases of PCNSL are DLBCLs, rare cases of peripheral T-cell lymphoma (PTCL), low-grade lymphoma, and BL also have been reported. In addition to B-cell markers, CD10 expression is observed in only 10% to 20% of cases, but *BCL6* expression is common (60% to 80%). Most cases (>90%) are of the activated B cell-like (ABC) subtype of DLBCL. Mutations of *CD79B*, *MYD88*, and *PIM1* are frequently observed. Amplifications of 9p24.1 are common and result in PD-L1 expression in the majority of cases. PCNSLs are rare and may occur in immunocompetent patients or in association with immunosuppression related to HIV infection or to organ and marrow transplantation. With the introduction of combination anti-

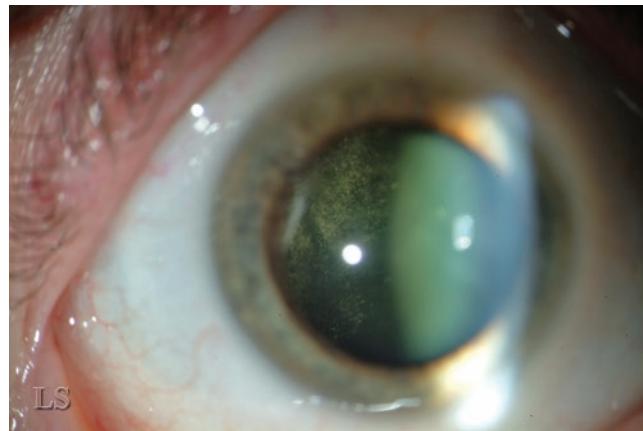


Figure 23-3 Intraocular large B-cell lymphoma on slit lamp examination.

retroviral therapy (cART), the incidence of PCNSL has decreased in HIV-infected persons. It appears, however, to be increasing in incidence in immunocompetent patients. In the latter group, the median age is 60 years, and it is discovered based on focal neurologic symptoms, personality changes, or symptoms of increased intracranial pressure. Ocular involvement can occur in 10% to 20% of patients and may be the sole site of disease at presentation (intraocular lymphoma). Concurrent leptomeningeal disease is found in 16% of patients through CSF analysis but occurs as the sole site in <5%. B symptoms, systemic symptoms of fever, night sweats, and weight loss, are extremely uncommon and should raise suspicions of systemic involvement.

Stereotactic-guided biopsy is the optimal method for diagnosing CNS lymphoma; gross total resection should be avoided. Steroids can interfere with pathologic diagnosis, and if they are started for neurologic symptoms, they should be withheld in patients with a presumptive radiologic diagnosis of CNS lymphoma to increase diagnostic biopsy yield. A contrast-enhanced MRI should be performed, along with lumbar puncture with CSF analysis. A slit-lamp examination should be performed to rule out concurrent ocular involvement. Staging should include full body PET/CT imaging, and, in men, testicular ultrasound because 4% to 12% of patients can have extraneuronal disease.

A prognostic scoring system has been developed in PCNSL, given the limitations of the Ann Arbor staging system and the IPI in this disease. The following five factors are associated with a poor prognosis: age older than 60 years; PS>2; elevated LDH; high CSF fluid protein concentration; and tumor location within the deep regions of the brain. Patients with 0, 1 to 4, or 5 of these factors have 2-year OS rates of 80%, 48%, or 15%, respectively.

The median survival after surgery alone is ~1–4 months. Whole-brain radiation is associated with a high response rate of 90%, but the median survival is only 12 months, and patients can develop significant cognitive dysfunction. CHOP has poor CNS penetration and should not be used in PCNSL. The exception is intravascular large B-cell lymphoma with CNS involvement because the mechanism of spread is likely different. Although there have been no randomized controlled studies to establish the best therapy, in retrospective analyses, outcomes are superior when high-dose methotrexate (HD-MTX) (3 to 8 g/m²) is incorporated into first-line regimens. With this approach, the 5-year OS is 30% to 40%. Some studies have added other CNS-penetrant chemotherapy drugs, such as cytarabine (ara-C). Rituximab therapy also appears to improve outcome. A phase 3 trial randomizing younger patients in a CR following HD-MTX to WBRT (45 Gy) or observation demonstrated an improvement in median PFS (18 months vs 12 months) but OS was similar, and toxicity was greater in patients who received radiation. For patients older than 60 years, the risks of neurotoxicity are considerable and manifests as dementia, ataxia, and incontinence, with a median time to risk-onset of approximately 1 year. Because of concerns of neurotoxicity even in younger patients, numerous studies are evaluating chemotherapy alone with CNS-penetrant drugs. The CALGB evaluated the combination of HD-MTX, temozolomide, and rituximab with consolidative HDC using ara-C and etoposide without WBRT; the 3-year PFS and OS were 50% and 67%, respectively. The international extranodal lymphoma study group (IELSG) conducted an important randomized trial, first randomizing patients to 1 of 3 induction arms: methotrexate and cytarabine (MA); methotrexate, cytarabine and rituximab (MAR); and methotrexate, cytarabine, thiotapec and rituximab (MATRix). For responding patients, a second randomization assigned patients to WBXRT versus HDC/ASCT. Results from the initial randomization showed that the MATRix combination resulted in the highest PFS and OS, followed by MAR, and then by MA. MATRix is therefore an appropriate standard of care in patients sufficiently fit to undergo this intensive chemotherapy approach.

The second randomization in the IELSG trial is based on increasing evidence of benefit for a thiotapec-based ASCT in CNS lymphomas. Several small phase 2 studies have evaluated upfront transplantation with cure rates ranging from 40% to 77% using a variety of lead-in chemotherapy and HDC regimens. In patients with relapsed or refractory primary CNS, HDC/ASCT is associated with a 2-year OS of 45%, a TRM of 16%, and severe neurotoxicity in 12%. The second randomization of the

aforementioned IELSG trial found identical 75% 2-year PFSs between HDC/ASCT and WBXRT but with significant neurotoxicity in the WBXRT arm, which therefore favors ASCT consolidation. Preliminary results of a GOELAMS study also comparing HDC/ASCT consolidation with WBXRT showed a PFS benefit favoring the transplantation arm, and a similar OS at 4 years. These data do support consideration of HDC/ASCT consolidation rather than WBXRT in young patients sufficiently fit to undergo transplantation.

For relapsed patients, methotrexate-based therapy is usually used again, particularly in those who have had a lengthy remission after initial therapy. Temozolamide alone or in combination with rituximab has shown an ORR of 26% and 53%, respectively, in relapsed and refractory patients. The combination of high-dose methotrexate, rituximab, and temozolamide (MRT) is well tolerated and associated with significant clinical activity in a small phase 2 study. CR was achieved in 14/18 (78%) patients at a median of 4 months. Three of 18 patients achieved a partial response (PR). At a median follow-up of 15.5 months from treatment initiation, 10/18 patients remain in CR and median PFS has not been reached. Novel biologically-directed therapies are also emerging in the management of relapsed/refractory PCNSL. The ABC subtype, which characterizes nearly all cases of primary CNS DLBCL, makes lenalidomide or ibrutinib appealing agents; both agents have demonstrated high response rates in small phase 2 studies. The 9p24.1 amplifications and PD-L1 expression make PD-1 inhibitors a potential option, and indeed small initial series have shown high and durable rates of remission. All three of these novel agents (lenalidomide, ibrutinib, and PD1 inhibitors) warrant ongoing study as single agents and in combination in the relapsed setting, as well as incorporation into frontline therapy.

Secondary CNS lymphoma

The rate of secondary involvement of CNS in aggressive lymphoma and lymphoblastic lymphoma, occurring in up to 30% of BL (see section Burkitt lymphoma in this chapter), varies by histology. In these highly aggressive lymphomas, CNS prophylaxis is routinely incorporated using intrathecal (IT) and systemic chemotherapy with or without cranial irradiation and has been shown to reduce the rate of CNS relapse and to prolong survival. Secondary CNS lymphoma may also be seen in DLBCL occurring in the brain parenchyma, leptomeningeal compartment, or both as an isolated event or with systemic relapse. Approximately 1% of patients with DLBCL have CNS involvement at diagnosis; the risk of subsequent CNS recurrence is approximately 4% but is increased in selected high-risk subgroups.

A number of extranodal sites have been associated with a higher risk of CNS relapse, including testis, kidney, and bone marrow (concordant). To create a robust risk model predictive of CNS recurrence risk, known as the CNS-IPI, the German High Grade Lymphoma Study group analyzed data on 2,164 patients treated with R-CHOP or R-CHOP-like therapy. The risk of CNS involvement was 3%, and adverse risk factors for CNS relapse on multivariable analysis were the 5 established IPI risk factors, plus renal or adrenal involvement. Using the total of these 6 risk factors present at diagnosis, three risk groups were created: low risk (0–1), intermediate risk (2–3), or high risk (4–6), with CNS relapse rates of 0.6%, 3.4%, and 10.2%, respectively. These data were validated in a 1,600-subject retrospective cohort from the British Columbia Cancer Agency and yielded similar results. Based on these data, patients with 4–6 CNS-IPI risk factors present at diagnosis would be classified as high risk for CNS recurrence and should be considered for CNS prophylaxis strategies.

Although these and other studies can effectively identify subgroups with a high risk for CNS disease, demonstrating a benefit for CNS prophylaxis has proven to be much more difficult in DLBCL. Furthermore, many of the studies evaluating CNS prophylaxis were published before the routine use of rituximab, which does appear to reduce risk, albeit to a modest degree. The RiCOVER-60 study evaluated 1,217 patients with aggressive lymphoma (81% DLBCL) and reported that 58 patients (4.8%) developed CNS relapse or progression with a median time of 8 months (1–39 months); the median survival from CNS relapse was only 3 months. Those patients who received rituximab had a lower risk of CNS relapse; however, the magnitude of difference was very small (3.6% vs 5.9%, $P=.043$). Other studies have confirmed that rituximab appears to reduce the risk of relapse, particularly in patients in a CR, suggesting the benefit, in part, may be due to better systemic disease control. The risk is not altogether eliminated, however, given the poor CNS penetration of rituximab. Modeled after BL and lymphoblastic lymphoma, intrathecal CNS prophylaxis often is administered to high-risk DLBCL patients, but the protective benefit is unknown, particularly because distribution within the leptomeningeal compartment is highly variable, and it offers no protection for the brain parenchyma which harbors the majority of DLBCL relapses in the CNS. Prophylactic use of HD-MTX (3.0 to 3.5 g/m²) with R-CHOP was evaluated retrospectively in 65 patients with high-risk DLBCL (elevated LDH, involvement of >1 extranodal sites, 4–5 Hollander criteria, high-risk location: bone marrow, testes, epidural, liver, adrenal, renal, orbit), and reported a low rate of CNS relapse (3%). Use of HD-MTX, however, is limited in elderly patients, particularly in those

with poor renal function. A similar strategy of systemic methotrexate prophylaxis is currently under evaluation in treatment of primary testicular DLBCL, a subset of DLBCL associated with a particularly high risk of CNS relapse in the study conducted by IELSG.

Despite the limitations and lack of evidence-based data to direct treatment, patients considered high-risk by the extranodal site involved or by the CNS-IPI model should be considered for CNS prophylaxis. Patients with any neurologic signs or symptoms should also be evaluated with diagnostic lumbar puncture including flow cytometry and brain MRI as appropriate. Our preferred method for CNS prophylaxis in eligible patients is systemic methotrexate 3.5 g/m² administered on day 15 of the 21-day R-CHOP-M cycle and usually administered with alternating cycles for a total of 3 methotrexate infusions, if tolerated. Intrathecal prophylaxis remains available for patients who are not considered candidates for systemic methotrexate therapy, such as patients who are very elderly or who have impaired renal function.

Burkitt lymphoma

BL is among the most aggressive of all human malignancies, with a rapid doubling time, acute onset, and progression of symptoms. Histologically, BL has a diffuse growth pattern of medium-size cells and a high mitotic rate; nearly 100% of cells are Ki-67 positive due to deregulated high-level expression of cMYC arising from reciprocal translocation with immunoglobulin-heavy (t8;14) or variant light-chain gene loci (t2;8 or t8;22) (Table 23-2). Additional mutations in the transcription factor that controls germinal center cell proliferation, *TCF3*, and its inhibitor, *ID3*, also cooperate with cMYC overexpression to drive proliferation. In conjunction with proliferation, there is also a high rate of cell death or apoptosis, and the dead cells are phagocytosed by histiocytes, which gives a “starry-sky” appearance at low power. The B cells are positive for CD19, CD20, BCL6, and CD10. BCL2 is usually negative, but rare weakly positive cases may be seen. Lack of TdT is critical to rule out ALL/lymphoblastic lymphoma. Recent studies have identified a subset of lymphomas that resemble BL by clinical course, morphology, immunophenotype, and gene expression but lack MYC rearrangements. This new provisional 2016 WHO entity has chromosome 11q alterations that appear to drive the Burkitt-like features (Table 23-3).

Originally described in its endemic form in African children presenting with jaw or facial masses, BL also occurs in sporadic form in the Western world, predominantly in children and young adults. It also is seen in HIV-infected patients. Nearly all endemic cases show evidence of EBV

infection and presence of the EBV genome, but such EBV infection is present in only a minority of sporadic cases.

Clinically, patients with BL frequently present with a bulky abdominal mass, B-symptoms and extranodal disease, including bone marrow involvement, is common (up to 70%). A leukemic phase can be seen, but pure acute leukemia is extremely rare. CNS dissemination, usually in the form of leptomeningeal involvement, may be present at diagnosis in up to 30% of patients; as a result, CNS chemoprophylaxis is integrated into the therapy for virtually all BL patients.

Therapy for BL must be instituted quickly because of the rapid clinical progression of the disease. Admission to hospital and tumor lysis precautions are essential and include vigorous hydration and allopurinol treatment with close monitoring of laboratory studies, including electrolytes and renal function. Recombinant uric acid oxidase (rasburicase) has been shown to be very effective in preventing uric acid nephropathy and its secondary metabolic complications. Multiple studies have shown that CHOP chemotherapy is inadequate for the treatment of BL, and intensified therapies result in higher cure rates. Multiagent combination chemotherapy, that includes high doses of alkylating agents and CNS prophylaxis, have improved the outcome for adults and children with the disease. Given the disease rarity, there are no randomized controlled treatment trials in adults comparing these approaches. Magrath et al, at the National Cancer Institute demonstrated a risk-adapted strategy that is useful for treatment stratification in both adults and children. Low-risk patients were those with a single extra-abdominal mass or completely resected abdominal disease and a normal LDH, and all other patients were considered high-risk. Low-risk patients received three cycles of cyclophosphamide, vincristine, doxorubicin, and methotrexate (CODOX-M), and high-risk patients received CODOX-M alternating with ifosfamide, etoposide, and cytarabine (IVAC) for a total of 4 cycles (i.e., 2 cycles each of CODOX-M and IVAC). All patients received intrathecal chemoprophylaxis with each cycle, and those with CNS disease at presentation received additional intrathecal therapy during the first 2 cycles. Approximately half of the patients were adults, and the 2-year EFS for all patients was 92%. Two other phase 2 studies have used the Magrath regimen with modifications. In a United Kingdom study, adult (age range, 16–60 years; median age, 26.5 years), non-HIV patients were treated with dose-modified CODOX-M (3 g/m^2) for 3 cycles if they were determined to be low risk (ie, normal LDH, PS of 0 or 1, Ann Arbor stage I or II, and no tumor mass $>10 \text{ cm}$), and all other patients were considered high risk and treated with alternating dose-modified CODOX-M/IVAC. The

2-year PFS for the patients with BL was 64%. A modified Magrath regimen was also studied in an older population of patients (median age, 47 years) with a reported 2-year EFS was 71%. Other therapeutic approaches have included the hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (HyperCVAD)/methotrexate-cytarabine regimen and ALL-type regimens. Retrospective analyses and a phase 3 trial evaluating the addition of rituximab to intensive chemotherapy for BL in adults demonstrated an improvement in PFS and establishes that rituximab should routinely be included in the treatment plan of these patients. Notably, the intensive regimens described above incur high rates of toxicity and are poorly tolerated by older adults. The results from 12 large treatment series (10 prospective and 2 retrospective) were combined to better determine outcome in patients with BL in patients older than 40 years. In total, 470 patients were identified, 183 of whom were older than 40 years. The median OS at 2 years with intensive short-duration chemotherapy in older patients was only 39% compared with 71% when all patients were considered, suggesting an unmet need in older BL patients. More recently, a phase 2 study at the National Cancer institute evaluated DA-EPOCH-R in 30 adult patients with BL. The treatment was well tolerated in older adults and produced a 5-year EFS of more than 90%. This approach has now been validated in a multicenter prospective phase 2 trial of 113 adults with BL treated at 22 centers in the US. At a median follow-up of 3 years, the EFS was 85.7%; treatment was equally effective in younger and older patients. Based on these data, DA-EPOCH-R can be considered an appropriate standard regimen for the treatment of BL and is preferred in older adults who do not tolerate intensive therapy well.

High-grade B-cell lymphoma, NOS

High-grade B-cell lymphoma, NOS, is a new diagnostic entity in the 2016 WHO classification that replaced the eliminated category of “B-cell lymphoma, unclassifiable, with features between DLBCL and BL.” Previously, B-cell lymphomas with morphologic and genetic features between DLBCL and BL, as well as a large proportion of DHLs (described above), were classified as “B-cell lymphoma, unclassifiable, with features between DLBCL and BL.” With the new classification scheme, DHLs are now classified as “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.” B-cell lymphomas with morphologic and genetic features between DLBCL and BL that lack the aforementioned gene rearrangements are now classified as “high-grade B-cell lymphoma, NOS.” Because this is a newly classified entity, the prognosis and

optimal management of these patients remains undefined. With the removal of the DHL patients from this category, the prognosis for the newly classified patients has likely improved. In the absence of data to guide therapy, most lymphoma specialists prefer more intensive strategies in these patients based on their high-risk histology, such as DA-EPOCH-R, which has been validated as effective in other high-grade B-cell lymphomas.

Immunodeficiency-associated lymphoproliferative disorders

Congenital or acquired immunodeficiency states are associated with an increased incidence of lymphoproliferative disorders. The WHO classification identifies four such categories: (i) primary immunodeficiency disorders, including Wiskott-Aldrich syndrome, ataxia-telangiectasia, common variable or severe combined immunodeficiency, X-linked lymphoproliferative disorder, Nijmegen breakage syndrome, hyper-IgM syndrome, and autoimmune lymphoproliferative syndrome; (ii) HIV infection; (iii) post-solid organ- or marrow-transplantation with iatrogenic immunosuppression; and (iv) methotrexate- or other iatrogenic-related immunosuppression for autoimmune disease. The lymphomas seen in these settings are heterogeneous and may include HL or, more commonly, aggressive NHL. Chédiak-Higashi syndrome also has been associated with an increased incidence of pseudolymphoma and true NHL.

Lymphoproliferative disorders associated with primary immune deficiencies (PIDs) most commonly are seen in pediatric patients and frequently are associated with EBV infection. Extranodal disease including the CNS is common. Lymphomas occurring in patients with PID do not differ morphologically compared with immunocompetent hosts. DLBCL is the most frequent histologic type, although T-cell lymphomas are more common in ataxia-telangiectasia. EBV-related lymphomatoid granulomatosis is associated with Wiskott-Aldrich syndrome. These malignancies respond poorly to standard therapy. Therapy depends on both the underlying disorder and the specific lymphoma subtype; allogeneic transplantation has been used successfully in some patients. Novel immunotherapeutic or pharmacologic strategies targeting EBV are being explored.

A newly recognized large B-cell lymphoma, that typically occurs in the setting of age-related or iatrogenic immunosuppression called EBV-positive mucocutaneous ulcer (Table 23-3), should be noted. Patients typically present with cutaneous or mucosal ulcers. The aggressive histologic features consist of large transformed EBV-positive B cells with Hodgkin-like features, which belies its indolent course with nearly all reported cases responding to reduction of immunosuppressive therapy.

HIV-associated lymphomas

HIV-associated lymphomas are most commonly DLBCL or BL, with rarer histologies including plasmablastic lymphoma and primary effusion lymphoma. Approximately two-thirds of DLBCL cases are EBV-associated. Outcomes for HIV-associated lymphomas were historically poor; however, since the advent of combination antiretroviral therapy (cART), outcomes in the modern era are similar to non-HIV lymphoma as long as the HIV is under good control and the CD4 count is over 200 cells/ μ L. Given the importance of optimal HIV control, cART is usually given concurrently with chemotherapy and in cooperation with the HIV specialist to avoid administration of antiretrovirals that can exacerbate chemotherapy toxicity.

Optimal chemotherapy and the role of rituximab with anthracycline combinations in HIV-associated DLBCL have been the subject of debate. One small randomized study conducted by the AIDS Malignancy Consortium (AMC 010) demonstrated no improvement in outcome comparing R-CHOP with CHOP and an increase in treatment-related infectious deaths. A subsequent analysis, however, indicated that the toxicity was higher in patients with a CD4 count <50. Furthermore, a phase 2 French study using R-CHOP in HIV-positive aggressive lymphomas (85% DLBCL) demonstrated a 2-year OS of 75% without an increase in life-threatening infections, which also may reflect the exclusion of poor-prognosis patients because patients could have no more than one of the following: CD4 < 100, PS >2, or prior AIDS. Thus, rituximab should be given to HIV patients if the CD4 count is >50, particularly given the strong evidence for improved survival in the HIV-negative setting. Concurrent administration of G-CSF is advised, given the high rate of infection in this population, and all patients should receive prophylaxis against *Pneumocystis jiroveci* infection. DA-EPOCH has been tested in HIV-aggressive lymphoma, the majority of which had DLBCL but with suspension of cART to avoid drug interactions. At 53 months, the PFS and OS were 60% and 73%, respectively. The AMC also tested EPOCH-R (AMC 034) in patients with HIV-positive, aggressive B-cell lymphomas with rituximab given concurrently or sequentially; the 2-year OS rates were 63% and 66%, respectively. The cART use was at the discretion of the treating physician but was used in the majority of patients. There was no greater risk of infection except in patients with a CD4 < 50. More recently, the NCI piloted a second-generation regimen short-course (SC)-EPOCH-RR (two doses of rituximab per cycle), with G-CSF support, in HIV-positive DLBCL patients with the goal of improving efficacy and reducing toxicity. Dose-dense rituximab was intended to enhance the chemo-

therapy and minimize the number of treatment cycles. A PET scan was performed after two cycles: if negative, only one more cycle was given; and if positive, two to three cycles were given. The 5-year PFS and OS were 84% and 64%, respectively. A pooled analysis of these two AMC trials with patients treated with R-CHOP or R-EPOCH suggested that patients receiving R-EPOCH had an improved EFS and OS after adjusting for the aaIPI and CD4 count. The TRM was greater in patients with CD4 counts <50 (37% vs 6%, $P=.01$) regardless of the regimen used. Despite the practice for many years at the NCI to suspend cART use during DA-EPOCH, modern cART regimens can safely be combined with chemoimmunotherapy; the combination is recommended by infectious-disease specialists, and should be considered the standard of care. Attention should be paid to certain classes of drugs that can cause drug-drug interactions, such as protease inhibitors, which may increase vincristine-associated toxicity. Among BL patients, both R-CODOX-M/R-IVAC and DA-EPOCH-R can be safely administered to HIV-BL patients. These patients should therefore be treated similarly to their HIV-negative counterparts.

Posttransplant lymphoproliferative disorders

Posttransplant lymphoproliferative disorders (PTLDs) occur as a consequence of immunosuppression in recipients of solid organ, bone-marrow, or stem-cell allografts. The risk is higher in solid-organ transplants that warrant a higher degree of immunosuppression (10%–25% in heart and lung transplants) than those that require a lower immune-suppression dosing (1%–5% kidney and liver transplants), but the most important risk factor for EBV-driven PTLD is pre-transplant EBV seronegativity. PTLDs are composed of a spectrum of disorders, ranging from EBV-positive infectious mononucleosis (early lesions) to polymorphic PTLDs, which most often are clonal, to full-blown monomorphic PTLDs that can be EBV-positive or EBV-negative and are further subdivided into B-cell lymphomas (common) with DLBCL being the most common, and T-cell lymphomas (rare); these are indistinguishable from their counterparts in immunocompetent hosts. HL-type PTLDs also can occur; however, indolent B-cell lymphomas arising in transplantation recipients are not among the PTLDs. EBV-negative PTLD has increased over the last decade and typically has a late onset (median time from transplantation to PTLD of 50–60 months vs 12 months in EBV-positive patients), a poorer response to therapy, and is more frequently monomorphic.

PTLDs have diverse clinical presentation depending on location. Extranodal involvement is common, particularly the gastrointestinal (GI) tract (~25%), lung,

skin, and bone marrow. Primary CNS lymphoma also can occur. The goal of treatment is to cure the lymphoma but also to preserve graft function. Although a significant minority (20–50%) of patients respond to a reduction in intensity of immunosuppressive drugs, most require additional systemic therapy, particularly for monomorphic or late PTLDs. Tolerance to chemotherapy is poor in PTLD patients, with treatment-related mortality reported to be as high as 31% in older series using CHOP chemotherapy. With historically poor tolerance to combination chemotherapy, single-agent rituximab has been explored in the first-line setting in PTLD. The ORR has ranged from 40% to 75%, and it is extremely well tolerated; however, remission duration may be short in many patients. In the first prospective phase 2 study, 43 PTLD patients who had failed to respond to a reduction in immunosuppression were treated with single-agent rituximab. The ORR was 44% at day 80 (CR 21%), and the 1-year OS was 67%. An updated analysis from this study evaluating 60 patients demonstrated an ORR of 59% (CR 42%), but the median PFS was only 6 months and the 2-year OS was 52%. Elevated LDH was predictive of disease progression as well as a shorter time from the date of transplant. Using a PTLD-adapted prognostic score incorporating age (>60 years), elevated LDH, and PS (>2), patients with a score of 0, 1, or 2/3 had 2-year OS estimates of 88%, 50%, and 0%, respectively, suggesting that single-agent rituximab may be suboptimal in high-risk groups. A subsequent phase II study, 152 patients with PTLD, who were treatment-naïve, were administered 4 weekly doses of rituximab, with subsequent therapy stratified based on CT scan response. Patients with a CR after rituximab alone received 4 additional doses of rituximab monotherapy at 21-day intervals, while patients without CR proceeded to 4 cycles of R-CHOP-21. Seventy percent of subjects achieved CR after rituximab monotherapy, with the remainder requiring R-CHOP. The 3-year PFS and OS in the entire population were 75% and 70%, respectively, suggesting that this sequential response-adapted treatment approach is a reasonable strategy and may avoid chemotherapy exposure in a significant proportion of patients. Reduced immunosuppression and single-agent rituximab are therefore reasonable first-line treatments in most patients with sequential therapy with R-CHOP reserved for those who do not achieve a CR after reduced immunosuppression and rituximab alone. For patients who present with very high-risk aggressive disease, R-CHOP can be considered frontline treatment with G-CSF support and inclusion of PJP prophylaxis.

Mantle cell lymphoma

MCL accounts for 6% of all NHLs and was characterized historically by poor outcomes and a short overall survival. But that was before treatments were developed specifically for this unique histology. Modern outcomes have markedly improved for younger and older patients alike, based on improved induction regimens and availability of targeted therapies at relapse.

MCL has distinctive clinical features including median age in the mid 60s, a striking male predominance, and a strong tendency to present with advanced-stage disease. Extramedullary involvement is common, including bone marrow and peripheral blood, plus a peculiar tendency to invade the GI tract, which may present as a distinctive syndrome of lymphomatous polyposis of the large bowel. Even patients without overt colonic polyposis frequently have subclinical GI epithelial invasion, which can be demonstrated on biopsy.

Cytologically, most MCLs consist of small lymphocytes with notched nuclei. The architectural pattern of the lymph node usually is diffuse but may show a vaguely nodular- or mantle-zone growth pattern. A spectrum of morphologic variants has been recognized which includes small cells, which are composed of small round lymphocytes and clumped chromatin, mimicking SLL/CLL, and a blastoid variant, which has a high mitotic rate and is clinically very aggressive. The immunophenotype of MCL is distinctive. Cases are typically CD5⁺, FMC7⁺, and CD43⁺ but CD10⁻ and CD23⁻ (Table 23-2). Some of the salient features that distinguish MCL from SLL or CLL are the expression of cyclin D1, SOX11, and FMC7 without CD23 expression (Table 23-2). Furthermore, MCL has a more intense IgM or IgD and CD20 expression than SLL/CLL. Virtually all MCLs carry the t(11;14)(q13;q32) on karyotypic analysis or by FISH. This reciprocal translocation juxtaposes the immunoglobulin heavy-chain locus and the cyclin D1 (*BCL1*) gene.

Biologic and clinical features have prognostic value in MCL. Cellular proliferation may be the most powerful predictor. cDNA microarray analysis has demonstrated that genes associated with cellular proliferation show striking variability among MCL cases, ranging from low to very high expression. Patients in the lowest quartile of expression have median survival times of 6–8 years, whereas patients in the highest expression quartile have survivals of <1 year. For clinical practice, Ki-67 staining can provide an estimate of proliferation. Three prognostic groups have been identified using cut points of <10% (best), 10% to 29% (intermediate), and >30% (worst). With regards to clinical factors, the IPI does not provide adequate prog-

nostic usefulness when applied to MCL, leading to the generation of an MCL-specific index. The MCL international prognostic index (MIPI) identified four clinical features, age, PS, LDH, and WBC, as independently associated with OS (Table 23-7). The MIPI score can separate patients into three risk groups and is quite valuable for characterizing patients in a clinical trial. Characterization is not always useful in clinical practice because older age and poor PS may classify a patient as “high risk,” but such a patient may not be a candidate for therapy intensification.

Of note, two types of clinically indolent MCL variants were recently recognized. One being in-situ mantle-cell neoplasia (Table 23-3), with the term neoplasia replacing lymphoma to emphasize the low rate of progression of this variant that is characterized by the presence of cyclin D1-positive cells in the mantle zones of otherwise normal follicles without evidence of nodal architectural disruption. Likewise, the second indolent MCL variant is a leukemic non-nodal MCL that is likely derived from a postgerminal-center B cell that usually lacks SOX11 expression. Patients with this variant typically present with peripheral blood lymphocytosis and splenomegaly without significant lymphadenopathy.

Management of newly diagnosed MCL

Initial therapy of MCL must be personalized to the patient, taking into account pathology, clinical presentation, age, and comorbidities. Patients with low-disease-burden asymptomatic MCL may safely be observed for a period of time, though most patients will require therapy. The indolent variants of MCL, which most commonly present with leukemic disease and splenomegaly with minimal adenopathy, are particularly good candidates for a period of observation, if asymptomatic. With patients in need of therapy, we typically divide them based on age (usually 65 or younger) and whether they are candidates for HDC/ASCT.

For younger patients with MCL, strategies incorporating rituximab, cytarabine, and HDC/ASCT consolidation have produced the best results with the longest PFS and OS. The Nordic Lymphoma Study Group phase 2 trial tested an intensive-induction immunochemotherapy with cycles of R-maxi-CHOP alternating with R-cytarabine, followed by in-vivo purge (with rituximab) and ASCT. The study was limited to patients younger than 65 years median age was 56 years. The ORR was 96%, and at 15 years of follow-up, the median PFS and OS were 8.5 years and 12.7 years, respectively. The European MCL Network has presented results of a large phase 3 randomized clinical trial with MCL patients <65 years. This trial compared the efficacy of six courses of R-CHOP

followed by HDC/ASCT vs alternating courses of R-CHOP/R-DHAP followed by a high-dose cytarabine containing HDC/ASCT. The study was designed to test the contribution of cytarabine in the management of younger MCL patients (median age 56 years). The 5-year PFS was significantly better in the cytarabine-containing arm (65% vs 40%). A recent prospective phase 3 trial from the French LYSA group administered 4 cycles of R-DHAP followed by HDC/ASCT in responding patients, who were then randomized to maintenance rituximab therapy vs no further therapy. The ORR and CRR after 4 courses of R-DHAP were 89% and 77%, respectively. Among randomized patients, the 4-year PFSs were 83% vs 64%, respectively, favoring maintenance rituximab. The 4-year OSs were also improved (89% vs 80%, respectively, $P=.04$), making maintenance rituximab the standard of care post HDC/ASCT in MCL.

Patients over the age of 60 have been evaluated in clinical trials which do not require HDC/ASCT. The European MCL Network conducted a trial for patients older than 60 years, who were assigned randomly to induction with R-CHOP or to the R-FC (rituximab, fludarabine, cyclophosphamide) regimen. Responding patients underwent a second randomization to maintenance therapy with rituximab (MR) or interferon- α (IFN α), each course given until progression. The median age of the 560 study participants was 70 years. Although response rates were similar between R-CHOP (86%) and R-FC (79%), the OS was significantly better in the R-CHOP arm (62% vs 47% at 4 years, $P=.005$). The inferior survival in the R-FC group was due to a combination of inferior disease control and increased death from infectious complications related to the immunosuppressive effects of fludarabine. Remission duration was significantly longer in the rituximab group than in the IFN group. At 4 years, 58% of the MR group remained in remission compared with 29% of the IFN group. Subgroup analysis indicated the benefit of MR was restricted to the R-CHOP-treated patients; the R-CHOP plus MR-treated patients experienced improved 4-year OS compared with R-CHOP- plus IFN-treated patients (87 vs 63%, $P=.005$), respectively. This trial indicates that R-CHOP followed by MR is a reasonable front-line approach for older MCL patients.

An additional phase 3 trial compared R-CHOP to an R-CHOP-like regimen (VR-CAP), where bortezomib replaced vincristine. The VR-CAP regimen was superior to R-CHOP for complete response rates (53% vs 42%), median PFS (24.7 months vs 14.4 months), and 4-year OS rate (64% vs 54%). The rates of neutropenia and thrombocytopenia were higher in the VR-CAP patients. Finally, the bendamustine-rituximab (BR) regimen also appears

to be a preferred alternative to R-CHOP. A large randomized trial compared BR with R-CHOP in patients with newly diagnosed indolent and MCL lymphoma. For the entire study population, BR was better tolerated than R-CHOP, with less alopecia, neutropenia, and infections. In the MCL patients ($n=93$), median age 70, BR was superior to R-CHOP for median PFS (35 months vs 22 months, $P=.006$). In a similarly designed trial was conducted in North America, MCL patients ($n=67$) comprised a subset of the study population. MCL patients assigned to BR were more likely to achieve complete remission than patients assigned to R-CHOP or R-CVP (50% vs 27%). Taken together, these studies suggest that the VR-CAP and BR regimens are better induction platforms than R-CHOP regimens in elderly patients with MCL, with BR being the best tolerated and most widely used. A small randomized trial evaluating MR after BR in MCL showed no improvement in this setting; therefore, BR without maintenance remains preferred when BR induction therapy is used.

Management of relapsed MCL

Younger patients relapsing after intensive therapies are candidates for allo SCT. The literature varies widely in the efficacy of this approach, but allo SCT does appear to have curative potential for a fraction of patients (25%-50%). A multicenter experience using a reduced-intensity conditioning (RIC) approach demonstrated 2-year EFS and OS rates of 50% and 53%, respectively. The 2-year transplant-related mortality rate was 32%, highlighting the high-risk/high-reward nature of allo SCT in relapsed MCL. For older patients, the BR regimen is highly active in relapsed MCL, with an ORR of 75% to 92% reported in two small studies. The proteasome inhibitor bortezomib is FDA-approved for relapsed MCL and has modest activity, with an ORR of 33% and a median PFS of 6 months. The mTOR inhibitor temsirolimus is European Union-approved for relapsed MCL, demonstrating an ORR of 22% and median PFS of 4.8 months in a pivotal study. Newer targeted therapies, however, are demonstrating improved clinical activity with decreased toxicity. The immunomodulatory agent lenalidomide is FDA-approved for recurrent MCL. In the EMERGE study ($n=134$), lenalidomide produced response rates of 28%. Although the median PFS was just 4 months, the median duration of response of 16.6 months indicated that responders can experience a durable benefit. Lenalidomide, which potentiates immune-effector cells, appears to be even more active when combined with rituximab. A phase 1/2 trial in relapsed MCL ($n=52$) reported an ORR of 57% and a median PFS of 11.1 months. Most promising of the new

agents are the Bruton tyrosine kinase (BTK) inhibitors, which interfere with signaling through the B-cell receptor pathway. In a single arm phase 2 trial ($n=111$) in relapsed/refractory MCL, the BTK inhibitor ibrutinib produced an ORR of 68%, CRR of 21%, and median PFS of 13.9 months. Ibrutinib was FDA-approved for patients with recurrent MCL in late 2013. A second generation BTK inhibitor, acalabrutinib has also been FDA-approved for relapsed/refractory MCL based on a 124-patient multi-center phase 2 study showing an ORR of 81% with CRR of 40% and a 12-month PFS of 67%. BTK inhibitors and lenalidomide are currently being explored in addition to up-front therapy and may ultimately decrease our reliance on intensive chemotherapy and stem-cell transplantation.

Peripheral T-cell lymphomas

PTCLs represent 10% to 15% of all NHLs in Western populations and are a heterogeneous group of mature T-cell neoplasms arising from postthymic T cells at various stages of differentiation. NK-cell lymphomas are included in this group because of the close relationship between these two cell types. The importance of the T-cell phenotype and the impact on prognosis are now well established but are relatively recent advances. A large retrospective study, the International T-Cell Lymphoma Project (ITLP), collected 1,153 cases of PTCLs from 22 centers from around the world and highlighted the geographic, clinicopathologic, and prognostic differences of this diverse group of diseases. There is a range of diseases among T- and NK-cell neoplasms, with most diseases behaving aggressively; however, a minority have a favorable prognosis or an indolent course (Table 23-3).

Indolent PTCLs

Mycosis fungoides and Sézary syndrome

In contrast to nodal NHLs, which are mostly B-cell derived, ~75% of primary cutaneous lymphomas have a T-cell phenotype and two-thirds are mycosis fungoides (MF) or Sézary syndrome (SS). MF is an epidermotropic, primary cutaneous T-cell lymphoma and represents the most common of all primary cutaneous lymphomas (50%). MF usually has an indolent course, but, like indolent B-cell lymphomas, it is considered incurable using conventional therapies. MF is limited to the skin in its early phases and appears as plaques or patches; but, with time, it evolves to diffuse erythroderma or cutaneous nodules or tumors, usually with associated adenopathy. The early-stage lesions appear characteristically in a bathing suit distribution and are often pruritic in nature. Extracutaneous disease can occur in advanced stages and may indicate histologic transformation. The histology varies with stage of the disease,

but epidermotropism is seen with typical plaques and intradermal collections of so-called Pautrier microabscesses. The T-cells are CD4⁺/CD8⁻, often with aberrant loss of one or more of the T-cell antigens CD2, CD3, CD5, and CD7. Progression to nodal disease, organ infiltration, and circulating clonal T-cells (SS) represents the advanced stage of the disease. A unique clinical staging system has been proposed by the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC) for MF and SS. The extent of cutaneous and extracutaneous disease is the most important prognostic factor in MF, with a 10-year disease-specific survival ranging from 97% to 98% for patients with limited patch/plaque disease (<10% of skin surface; stage I) to 20% for patients with lymph-node involvement.

SS is a distinct disorder characterized by erythroderma, generalized lymphadenopathy, and the presence of Sézary cells in the skin, lymph nodes, and peripheral blood. It is associated with an aggressive course with a 5-year OS rate of 20% to 30% with lower rates seen with high Sézary cell counts.

Because MF is incurable and the use of early therapy does not affect survival, a nonaggressive approach is recommended. Patients with stage IA disease may be managed expectantly with careful surveillance. If treatment is needed, topical steroids or topical nitrogen mustard, electron-beam radiotherapy, or cutaneous photochemotherapy with oral psoralen plus ultraviolet A (PUVA) typically are employed. Phototherapy with PUVA or ultraviolet B (UVB) is recommended for more widespread disease. Low-dose radiotherapy can be helpful to improve symptoms and cosmesis. Patients with progressive disease and those with systemic dissemination may be appropriately treated with methotrexate or corticosteroids, although responses are usually brief.

Combination chemotherapy regimens are not particularly effective and provide only transient responses. Single-agent treatments are preferred, particularly with slowly progressive disease, because of a high risk of myelosuppression and infection and only modest response durations seen with combination chemotherapy. Gemcitabine (ORR 48%-75%), pentostatin (ORR 28%-71%), and liposomal doxorubicin (ORR 56%-88%) have single-agent activity. Alternatively, IFNa, bexarotene, vorinostat, romidepsin, and brentuximab vedotin all have efficacy in advanced-stage MF and SS. Brentuximab vedotin is preferred in CD30-positive cases based on the international phase 3 ALCANZA trial where 131 patients with CD30-positive relapsed/refractory MF or CTCL were randomized between the anti-CD30 antibody drug conjugate brentuximab vedotin, or the in-

vestigator's choice of oral methotrexate or oral bexarotene. Patients treated with brentuximab vedotin had significant improvement in the primary endpoint of objective response lasting at least 4 months (56.3% vs 12.5%), resulting in FDA-approval for brentuximab vedotin in this indication.

Bexarotene is an oral retinoid and is FDA-approved for cutaneous T-cell lymphoma (CTCL). In a multicenter trial of 94 patients with advanced stage MF/SS, the ORR was 45% but with only 2% CRs. The common toxicities are hypertriglyceridemia (82%) and central hypothyroidism (29%). The histone deacetylase (HDAC) inhibitors, vorinostat and romidepsin, are both approved for the treatment of CTCLs. Vorinostat is available orally and has an ORR of ~30% and a median duration of response (DOR) of ~6 months. A phase 2 trial with romidepsin demonstrated an ORR of 35% (CR 6%) with a median DOR of 15 months in one study and 11 months in another. Side effects that are common with histone deacetylase (HDAC) inhibitors are fatigue, nausea, vomiting, neutropenia, and thrombocytopenia. Prolonged QT syndrome also can occur, and thus electrolytes should be monitored closely, and an electrocardiogram should be performed in high-risk patients during therapy. Alemtuzumab, the humanized monoclonal antibody targeting CD52, also has been used in MF and SS with some success; however, patients are at high risk of opportunistic infections. Studies evaluating low-dose alemtuzumab (10 mg thrice weekly) have been similarly effective with reduced toxicity, and should be preferred. Small studies also report single-agent activity for lenalidomide (ORR 28%) and low dose pralatrexate given at 15 mg/m² for 3 of every 4 weeks (ORR 45%).

Allogeneic transplantation has been explored in selected cases of MF and SS. The European Group for Blood and Marrow Transplantation recently reported a multi-institutional retrospective study evaluating allo SCT (myeloablative and RIC) in 60 patients with MF ($n=36$) or SS ($n=24$). Almost half had refractory disease at the time of allo SCT; the median number of prior regimens was four. With a median follow-up of 3 years, the 3-year PFS and OS were 34% and 53%, respectively, with higher survival rates observed in the RIC group (3-year PFS 52% vs 29%, $P=.006$).

Large-cell transformation in MF is defined as large cells in >25% of the infiltrate or as cells forming microscopic nodules. The incidence ranges from 8% to 39% and typically is associated with a poor prognosis, but there have been some long-term survivors. One study evaluated 100 cases of transformed MF; the median survival was 2 years with a 5-year OS and a disease-specific survival (DSS) of 33% and 38%, respectively, compared to MF patients without transformation. The factors associated with a poor

DSS were CD30-negative status, folliculotropic MF, generalized skin lesions, and extracutaneous transformation. Those cases with zero factors had a 2-year DSS of 83% compared with 14% to 33% in patients with three or four factors. The optimal management is unclear, but for young patients, systemic chemotherapy should be used and autologous or allogeneic transplantation should be considered particularly with high-risk disease. Consolidative radiation may be an option in local transformations.

Primary cutaneous ALCL

Primary cutaneous ALCL (C-ALCL) is part of a spectrum of diseases belonging to the category of primary cutaneous CD30⁺ T-cell lymphoproliferative disorders that also includes lymphomatoid papulosis and "borderline" cases that have overlapping features of both disorders. C-ALCL is the second most common type of CTCL. Patients are typically older males (median age 60 years), presenting with a solitary nodule with multifocal disease occurring in only 20% of patients. Partial or complete spontaneous regression occurs in ~25% of cases. C-ALCL must be distinguished from systemic ALCL with secondary cutaneous involvement through staging procedures.

The outcome is very favorable with a 10-year DSS of 95%. It is notable that patients with localized C-ALCL with one draining lymph node involved have a similarly good prognosis. For localized C-ALCL, radiation is the preferred therapy. Progression to systemic involvement can occur in a minority of cases. For more advanced-stage cases, the best management is unclear. An argument can be made to treat minimally symptomatic patients conservatively with palliative dose radiotherapy just to the few most prominent lesions, but for patients where systemic therapy is required, brentuximab vedotin is preferred based on the aforementioned data for this agent in CD30⁺ CTCL.

T-cell large granular lymphocytic leukemia and chronic lymphoproliferative disorder of NK cells

T-cell large granular lymphocytic leukemia (T-LGL) is defined by a persistent (>6 months) increase in the number of peripheral-blood large granular lymphocyte cells without an identifiable cause. The lymphocytosis is usually between 2×10^9 and $20 \times 10^9/L$. The malignant T-LGL cells are positive for CD3 and CD8, and CD57/CD16 are expressed in most cases, but CD56 is negative. It may arise de novo or in the context of rheumatoid arthritis or other autoimmune disorder. T-LGL must be distinguished from reactive LGL populations which may be seen in the setting of chronic viral infections or autoimmune conditions. Assessment of clonality with T-cell receptor PCR is often helpful

in establishing the diagnosis. Most cases have an indolent clinical course, and T-LGL is usually not considered a life-threatening disease; however, rare cases with an aggressive course have been described. Chronic lymphoproliferative disorder of NK cells (CLPD-NK) have similar clinic features and indolent course, but the neoplastic cells have an NK cell immunophenotype with expression of CD16 and CD56, variable expression of CD2, CD5, and CD7, and lack of surface CD3. STAT3 mutations are found in about 30% of both T-LGL and CLPD-NK. Of note, T-LGL and CLPD-NK should be distinguished from aggressive NK-cell leukemia, which have a fulminant aggressive course (see the section Aggressive NK-cell leukemia). In T-LGL and CLPD-NK, moderate splenomegaly is the most common clinical finding, and lymphadenopathy is rare. Severe neutropenia with or without anemia is common, and pancytopenia may be seen. A variety of autoimmune disorders, including hemolytic anemia, thrombocytopenia, and pure red blood cell aplasia, also may occur. If treatment is required for cytopenias, immunomodulatory agents, such as low-dose methotrexate, cyclophosphamide, and cyclosporine A, are often effective, and corticosteroids can provide a useful adjunct. Responses can take up to 4 months, and longer therapy often is needed to maintain the response. Weekly low-dose oral methotrexate is most commonly used as initial therapy, though oral cyclophosphamide at a dose of 50 to 100 mg by mouth daily has anecdotally appeared to be more effective in anemia-predominant disease. Purine analogs have been used in highly refractory patients. Splenectomy may be useful in selected cases with an accompanying splenomegaly, refractory cytopenias, or autoimmune hemolytic anemia or thrombocytopenia. The anti-CD52 monoclonal antibody alemtuzumab can be used in select cases.

Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract

Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract is a clonal proliferation typically involving CD8-positive T cells that infiltrate the lamina propria of multiple sites in the small intestine and colon. Patients typically present with abdominal pain, dyspepsia, diarrhea, and weight loss. Biopsies demonstrate a lymphoid infiltrate in the lamina propria that shows little histologic evidence of epithelial invasion, and, accordingly, patients generally have an indolent relapsing clinical course. Response to chemotherapy is poor, but patients have prolonged survival with persistent disease.

Primary cutaneous acral CD8⁺ T-cell lymphoma

Primary cutaneous acral CD8⁺ T-cell lymphoma is a rare cutaneous lymphoma that typically occurs at acral sites,

such as the ear, nose, or soles of the feet as an isolated papule or nodule with a history of slow growth. Histologically, there is a dermal proliferation of intermediate-sized atypical CD8⁺T cells that lacks aggressive features, such as angiodestruction and necrosis, and spares the epidermis. Local excision or radiotherapy typically leads to complete remission.

Aggressive PTCLs

Adult T-cell leukemia/lymphoma

Adult T-cell lymphoma/leukemia (ATLL) is caused by infection with HTLV-1 and occurs in areas of endemic infection (eg, the Caribbean basin and southwestern Japan). The cumulative incidence of ATLL among HTLV-1 carriers is 2.5% in Japan. The virus can be transmitted in breast milk and blood products. The malignant cells have a distinct cloverleaf appearance and are CD7⁻, and most are CD4⁺/CD8⁻ and CD25⁺. The following clinical variants have been recognized: (i) acute type with a rapidly progressive clinical course including bone-marrow and peripheral-blood involvement, hypercalcemia with or without lytic bone lesions, skin rash, generalized lymphadenopathy, hepatosplenomegaly, and pulmonary infiltrates; (ii) lymphoma type with prominent adenopathy but lacking peripheral blood involvement but also associated with an aggressive course; (iii) chronic type with lymphocytosis and occasionally associated with lymphadenopathy, hepatosplenomegaly, and cutaneous lesions but having an indolent course; and (iv) smoldering type with <5% circulating neoplastic cells, skin involvement, and prolonged survival. The chronic and smoldering forms can progress to the acute form after a variable length of time. In the ITLP, 126 patients (9.6% of all PTCLs) were identified with the acute (13%) or lymphoma-type (87%) ATLL. Opportunistic infections are common, and *Strongyloides* serology is recommended before starting therapy.

Survival times in the acute and lymphomatous variants are ~6 and ~10 months, respectively. The median survival for the chronic form is 2 years. The 4-year OS for the acute, lymphoma, chronic, and smoldering types has been reported to be 5%, 5.7%, 27%, and 63%, respectively. Asymptomatic patients with the smoldering or chronic type ATLL can be monitored closely. For young, fit patients with the acute and lymphoma subtypes, the intensive chemotherapy regimen incorporating VCAP (vincristine, cyclophosphamide, doxorubicin and prednisolone)/AMP (doxorubicin, ranimustine, prednisolone)/VECP (vindesine, etoposide, carboplatin, prednisolone) may be considered. The Japan Clinical Oncology Group (JCOG) reported a phase 3 trial comparing the dose-intensive regimen VCAP/AMP/VECP versus CHOP-14 alone that showed a more favorable CR rate (40% vs 25%, $P=0.02$) and 3-year OS (24% vs 13%) that was significant after adjusting for prognostic

factors but only for the one-sided *P*-value (*P*=0.028). The median survival for the intensive regimen was just over 1 year, but toxicity was high (grade 4 neutropenia in 98% and grade 3/4 infections in 32%). Thus, this regimen should be used only in carefully selected patients, particularly with the lymphoma subtype. Relapse rates remain high, and relapsed patients should be considered for transplantation.

A number of phase 2 studies evaluating the use of the antiretroviral zidovudine (AZT) and IFN in untreated patients have found response rates up to 92% and a median OS of 11 months. For patients with the leukemia subtype, these results are superior to what is achieved with combination chemotherapy, though the benefit appears minimal in the lymphoma subtype. For patients with the chronic and smoldering types, a meta-analysis demonstrated 100% OS after 10 years with this approach.

Chemokine receptor 4 (CCR4) is expressed in ~90% of cases of ATL. Mogamulizumab (KW-0761) is a humanized monoclonal antibody targeting CCR4; a phase 2 study demonstrated an ORR of 50%, including eight CRs, in 27 treated patients. The median PFS and OS were 5.2 months and 13.7 months, respectively. The most common side effects were lymphopenia (96%), neutropenia (52%), thrombocytopenia (52%), infusion reaction (89%), and skin rashes (63%).

PTCL, not otherwise specified; systemic anaplastic large cell lymphoma; and angioimmunoblastic T-cell lymphoma

PTCL-NOS, systemic anaplastic large-cell lymphoma (ALCL), and angioimmunoblastic T-cell lymphoma (AITL) are the most common subtypes of PTCL encountered in North America and represent 66% of all PTCL cases.

PTCL-NOS

PTCL-NOS is the most common subgroup of PTCLs, accounting for up to 30% of cases worldwide. PTCL-NOS is the default PTCL category for any mature T-cell neoplasm that does not fit into any of the specified categories in the WHO classification. Patients typically present with advanced-stage disease, and the 5-year OS is 20% to 30% in most series. The morphologic spectrum of PTCL-NOS is wide, including the histiocyte-rich lymphoepithelioid, or Lennert, lymphoma. Typically, the neoplastic cells are CD4⁺/CD8; CD5 and CD7 frequently are lost, and ~30% are CD30⁺.

Treatment approaches in PTCL have paralleled those for DLBCL; as a result, CHOP-like therapy is routinely employed as frontline therapy. The DSHNHL group retrospectively analyzed the outcome of PTCL patients (*n*=331) that had been enrolled in phase 2 or phase 3 aggressive

lymphoma studies and evaluated the impact of etoposide. In patients younger than 60 years with a normal LDH, EFS was extended with etoposide (*P*=.003), whereas OS did not improve significantly (*P*=.176). The addition of etoposide appeared to have the greatest impact in the favorable group of patients with ALK-positive ALCL (3-year EFS 91% vs 82%, *P*=.012). In patients with PTCL-NOS, ALK-negative ALCL, and AITL, there was a trend toward improved 3-year EFS (61% vs 48%; *P*=.057), with no OS difference observed; however, patient numbers were small. On the basis of these data, CHOEP may be considered as initial therapy in younger patients. For sufficiently young and fit patients, upfront consolidation with HDC/ASCT is generally considered (see Transplantation in PTCL below).

Newer chemotherapies and targeted agents are available for relapsed disease. Pralatrexate is a novel folate analogue that has enhanced uptake and cellular retention compared with MTX. Early studies suggested a sensitivity of TCLs over BCLs. The phase 2 PROPEL study evaluated pralatrexate (with vitamin B₁₂ and folate) in relapsed/refractory PTCLs and demonstrated an ORR 29% (CR 11%), a median PFS of 3.5 months and a median duration of response (DOR) of 10.5 months. The main toxicities were mucositis, thrombocytopenia, and neutropenia. These results led to FDA approval of pralatrexate in September 2009 for the treatment of relapsed/refractory PTCL. Studies combining pralatrexate with other agents in the upfront and relapsed settings are ongoing.

Romidepsin is an HDAC-inhibitor that has been evaluated in CTCLs and PTCLs. A phase 2B registration study evaluated romidepsin in 130 patients with relapsed or refractory PTCL. The ORR was 25% (CR 15%), median DOR was 17 months, and median PFS was 4 months, leading to FDA approval in 2011. Side effects were as previously described in the CTCL studies. A phase 1b study is ongoing combining CHOP with romidepsin for the primary treatment of PTCL.

Belinostat is another HDAC-inhibitor that has demonstrated responses in relapsed or refractory PTCL in a phase 2 trial. Belinostat was granted approval by the FDA for the treatment of patients with PTCL who have received at least one prior therapy. A phase 2 trial (BELIEF trial) of belinostat in 120 patients with PTCL reported overall and complete remission rates of 26% and 11%, respectively, with a median DOR of 13 months.

CD30 is expressed uniformly in ALCL but is also highly restricted, making it an attractive target in this disease. Studies with the nascent anti-CD30⁺ in relapsed systemic ALCL were largely disappointing, however. Therefore, an antibody-drug conjugate (ADC), brentuximab vedotin, was

developed to enhance tumor activity. The ADC conjugates the CD30 monoclonal antibody to the microtubule inhibitor, monomethyl auristatin E (MMAE), by an enzyme-cleavable dipeptide linker. Following binding to CD30⁺ and uptake into the cell, MMAE is released and interferes with tubulin formation. A phase 2 study, recently reported in relapsed or refractory systemic ALCL, demonstrated an ORR of 86% (CR 57%), median DOR of 12.6 months, and a median PFS of 13.3 months, which also prompted FDA approval of brentuximab vedotin for ALCL in 2011. The main side effect of brentuximab vedotin is peripheral neuropathy. Studies are ongoing evaluating brentuximab vedotin in the up-front setting with CHOP, omitting vincristine because of overlapping toxicity.

Angioimmunoblastic T-cell lymphoma and nodal lymphomas of T follicular helper (TFH) cell origin

AITL is a well-defined, distinct PTCL subtype with unique pathobiologic features. Key morphologic findings of AITL include an expanded CD21⁺ follicular dendritic-cell network and prominent arborizing high-endothelial venules (HEV). The neoplastic cells in AITL are mature CD4⁺/CD8⁻ T-cells, expressing most pan-T-cell antigens. EBV-positive B cells are seen in most cases, and EBV-positive DLBCL has been reported. It appears that the cell of origin is the follicular helper T-cell with T-cells CD10⁺, BCL6⁺, and CXCL13⁺, and derivation also is supported by gene-expression profiling studies. Sequencing studies have shown this PTCL subtype to be enriched for mutations of *TET2*, *IDH2*, *DNMT3A*, *RHOA*, and *CD28*.

Patients are typically in their sixth or seventh decade and have advanced-stage disease, often with B-symptoms and hepatosplenomegaly. AITL was originally believed to be a form of immune dysregulation, with polyclonal gammopathy and other hematologic abnormalities (Coombs-positive hemolytic anemia) reflecting B-cell dysregulation. Opportunistic infections can occur because of the underlying immunodeficiency.

Survival is similar to that in PTCL-NOS (5 year ~30%); however, a small proportion of patients may have a more indolent course. CHOP or CHOEP is typically used as primary therapy, and, although the response rate is high, relapse is common and infectious complications are problematic. GELA evaluated AITL patients enrolled in different therapeutic protocols and found no improvement of survival with any therapy, including HDC/ASCT. Because of poor outcomes using conventional therapy, immuno-modulatory agents, including cyclosporine, lenalidomide,

thalidomide, and interferon, also have been explored. A retrospective study evaluating cyclosporine in relapsed or refractory AITL demonstrated an ORR of 67% and a median DOR of 13 months. Among patients with relapsed disease, the HDAC inhibitors appear to have improved activity in AITL relative to other PTCL subtypes, making these agents appealing for patients failing frontline chemotherapy. Similarly, brentuximab vedotin has produced encouraging response rates in relapsed AITL, where the infiltrating B immunoblasts are usually CD30⁺.

Follicular T-cell lymphoma and nodal PTCL with TFH phenotype are also distinct nodal T-cell lymphomas derived from the same TFH cell as AITL. They share recurrent genetic abnormalities with AITL, including *TET2*, *IDH2*, *DNMT3A*, *RHOA*, and *CD28* mutations as well as t(5;9) *ITK-SYK* fusion. The clinical course of these rarer variants is not yet well characterized, but they appear to have an aggressive clinical course similar to AITL.

Systemic anaplastic large-cell lymphoma

ALCL is composed of large CD30⁺ anaplastic cells with a predilection for a sinusoidal and cohesive growth pattern. In the WHO classification, systemic ALCL is separated from primary cutaneous ALCL. Systemic ALCL cases are divided into two groups: ALK-positive and ALK-negative. (Table 23-3). Cases of ALK-positive ALCL are associated with a characteristic chromosomal translocation, t(2;5) (p23;q35), resulting in a fusion gene, *NPM-ALK*, encoding a chimeric protein with tyrosine kinase activity. With the availability of antibodies to the ALK protein, ALK expression can be demonstrated in 60% to 85% of all systemic ALCL, with higher frequencies seen in the pediatric and young adult age-groups.

ALK-positive ALCL. Morphologically, ALK-positive ALCL has pathognomonic “hallmark cells” recognized by their eccentric, horseshoe, or kidney-shaped nuclei. In addition to strong expression of CD30, ALK-positive ALCL is usually positive for epithelial membrane antigen (EMA) and cytotoxic markers (TIA1, granzyme B, and perforin). Several studies have established that patients with ALK-positive ALCL have a more favorable prognosis with anthracycline-based chemotherapy than patients who have ALK-negative ALCL and other PTCLs, as well as DLBCL, at least in the prerituximab treatment era. The improved outcome, at least in part, is related to the young age and low risk features often present at presentation. The ITLP confirmed the superior outcome of ALK-positive ALCL (5-year FFS, 60%; 5-year OS, 60%) compared with ALK-negative ALCL (5-year FFS, 36%; 5-year OS, 49%). If the

comparison is confined to patients younger than 40 years, however, there was no difference in survival. Similar findings were reported from a retrospective analysis of patients with ALCL enrolled on GELA studies, which reported that, in patients younger than 40 years, there was no impact of ALK status on PFS or OS.

Given the favorable outcome with anthracycline-based chemotherapy, CHOP-like therapy is considered the standard therapy for ALK-positive ALCL. A subset analysis of ALK-positive ALCL patients treated in prospective studies from the German High Grade Lymphoma Study Group has identified a particularly favorable outcome among patients treated with CHOEP (3 year EFS, 92%). More recently, a randomized phase 3 trial evaluated the upfront addition of BV (brentuximab vedotin) to CHP (cyclophosphamide, doxorubicin and prednisone), compared to standard CHOP, in CD30⁺T-cell lymphomas (70% were ALCL). 452 patients were randomized, and the study found an improved PFS favoring the BV-CHP arm (hazard ratio 0.71, p=0.01). Overall survival was similarly improved among BV-CHP treated patients (hazard ratio 0.66, p=0.024). Based on these data, BV-CHP can now be considered standard frontline therapy for ALK+ or ALK- ALCL.

Crizotinib and other small molecule inhibitors of the ALK tyrosine kinase, FDA-approved for treatment of ALK-positive non-small-cell lung cancer, have also demonstrated remarkable clinical activity in patients with multiply relapsed ALK-positive anaplastic large-cell lymphoma (ALCL) and may be considered in patients with disease that has been refractory to both chemotherapy and brentuximab vedotin.

ALK-negative ALCL. Patients with ALK-negative ALCL tend to be older at presentation; the clinical presentation is similar to PTCL-NOS, but sites of extranodal disease may vary. Histologically, ALK-negative ALCL is not reproducibly distinguished from the so-called common pattern of ALK-positive ALCL except that it lacks the ALK protein. ALK-negative ALCL has been difficult to define, in part, due to a lack of uniformly applied diagnostic criteria across studies. Previously, it was argued that ALK-negative ALCL had an outcome similar to that of PTCL-NOS and the two should be grouped together. In recent years, it has become clear that they differ not only pathologically and genetically but also prognostically. The ITLP compared the outcome of ALK-negative ALCL with PTCL-NOS and established that ALK-negative ALCL had a more favorable 5-year FFS (36% vs 20%, P=.012) and OS (49% vs 32%, P=.032). Gene-

expression studies have shown that ALK-negative ALCL has a signature distinctly different from PTCL-NOS and similar to that of ALK-positive ALCL. A subset of ALK-negative ALCL cases carry *DUSP22-IRF4* rearrangements and appear to have superior outcomes, similar to that of ALK-positive ALCL, while another subset carrying *TP63* rearrangements have poor outcomes. These data confirm that ALK-negative ALCL should be considered distinct from both ALK-positive ALCL and PTCL-NOS. Although the survival for ALK-negative ALCL is more favorable than for PTCL-NOS, it is still poorer than for ALK-positive patients, except in patients carrying the *DUSP22-IRF4* rearrangement. Initial therapy is with the BV-CHP regimen based on the aforementioned randomized trial showing superiority over CHOP. Upfront consolidation with HDC/ASCT is generally considered for ALK-negative patients, particularly those lacking the *DUSP22-IRF4* rearrangement (see the section on transplantation below). Brentuximab vedotin is highly effective in the relapsed/refractory setting, if it had not been incorporated with frontline therapy.

Breast-implant-associated ALCL. ALCL associated with implants typically presents as an unexplained seroma or capsule thickening. The lymphoma typically involves the capsule only, without invasion of the breast tissue or formation of discrete mass lesions. Almost all cases are localized. The tumor cells are CD30⁺ and ALK negative. The neoplastic cells float in the effusion fluid or become embedded tissue; importantly, however, breast parenchyma usually is not involved, and the ALCL cells infiltrate the cavity containing the implant rather than the breast tissue directly. Breast-implant-associated ALCL has been associated with both silicone and saline implants, but, importantly, it occurs almost exclusively in implants with a textured, as opposed to a smooth, surface. A total capsulectomy should be performed, and, because bilateral cases have been reported, removal of the uninvolved breast implant is generally considered. The growing body of literature supports that ALK-negative ALCL in this setting appears to have an indolent clinical course with a favorable prognosis, and most patients can be observed following removal of the implant and capsule and will not require adjuvant therapy. Recent reports suggest similar survival rates compared with those who received chemotherapy or radiation; however, rare aggressive cases have been reported where chemotherapy may be required. Cases that have identified a distinct breast mass may be better classified as a typical systemic ALK-negative ALCL and may be treated accordingly.

Extranodal NK-/T-cell lymphoma, nasal type

Extranodal NK-/T-cell lymphoma, nasal type, display great variation in racial and geographic distribution, with the majority of cases occurring in Asia. Patients are typically males aged 40 to 50 years. The tumor cells show angioinvasion and prominent necrosis. The designation NK/T is used to reflect the fact that, although most cells are NK-cell derived (CD2⁺, CD56⁺, CD3 [cytoplasmic]⁺, EBV⁺), rare cases with identical clinical and cytologic features exhibit an EBV-positive or CD56⁻, cytotoxic T-cell marker positive (TIA1, perforin, and granzyme B). Circulating EBV in the peripheral blood can often be detected, providing another method of disease monitoring. Most cases remain localized but may be extensively locally invasive, with <20% of patients presenting with advanced-stage disease. Despite the predominant nasal location, spread to the CSF is uncommon. Most occur in the nasal region, but identical tumors also can occur at extranasal sites, such as skin, soft tissue, GI tract, and testis (ie, extranasal). It appears that cases involving extranasal regions may have a more aggressive course. From the ITLP, the 5-year OS for stage I/II NK-/T-cell lymphomas were ~50% and 15% for nasal and extranasal sites, respectively, and the corresponding estimates for stage III/IV patients were 30% and <10%. The IPI does not stratify patients well because most have localized disease and often with good PS. A Korean index, using B symptoms, stage (I/II vs III/IV), regional lymph nodes, LDH, and PS, appears to be more useful in prognostication, particularly for the low- and low-intermediate IPI cases and may help to guide treatment decisions. Patients fall into four risk groups with widely disparate outcomes: group 1: no RF, 5-year OS ~81%; group 2: 1 RF, 5-year OS ~64%; group 3: 2 RF, 5-year OS ~34%; and group 4: 3 or 4 RF, 5-year OS 7%. Risk factors identified in other studies have also included local tumor invasion (tongue or skin), high Ki-67, or EBV DNA titer >6.1 × 10⁷ copies/mL.

Radiotherapy is important in the management of patients with localized NK-/T-cell lymphoma with more favorable outcomes observed using high doses of radiotherapy (50–60 Gy) early in the frontline setting. Use of platinum-based concurrent chemotherapy as a radiosensitizer appears highly effective and may allow for the use of lower, less-toxic doses of radiation. Furthermore, because systemic relapse can occur with single-modality radiotherapy, other novel combinations are being tested. The outcome with CHOP has been disappointing, and it has been speculated that this may be due to overexpression of p-glycoprotein expression conferring multidrug resistance. Concurrent radiation (40 Gy) and cisplatin, followed by three cycles of VIPD (etoposide, ifosfamide, cisplatin), was evaluated in stage IE/IIE nasal NK-/T-cell

lymphoma. In this highly selected population, the CR rate was 83% and the 3-year PFS was 85%. Similarly, concurrent radiotherapy (50 Gy) and DeVic chemotherapy (dexamethasone, etoposide, ifosfamide, carboplatin) was evaluated with good results in a phase 1/2 trial in localized nasal NK-/T-cell lymphoma (CR 77%, 2-year PFS 67%). In the absence of a randomized trial, limited-stage patients may be treated with high-dose radiotherapy alone (>50 Gy) for stage 1 patients without risk factors or concurrent chemoradiotherapy (stage 1 or 2) using either of the noted regimens for localized NK-/T-cell lymphoma.

For advanced-stage disease, L-asparaginase has emerged as an active agent in NK-/T-cell lymphomas with an ORR of 87% (CR 50%) in relapsed or refractory patients. Antithrombin levels require close monitoring. A phase 2 study, evaluating L-asparaginase in combination with MTX and dexamethasone (AspaMetDex) in previously treated patients, demonstrates an ORR of 78% (CR 61%) and a median DOR of 12 months. A phase 2 study evaluating the SMILE regimen (steroid, methotrexate, ifosfamide, L-asparaginase, etoposide) in 38 patients with newly diagnosed stage IV or relapsed or refractory NK-/T-cell lymphoma demonstrated an ORR after two cycles of 79% (CR 45%); 19 patients subsequently underwent SCT. The 1-year OS rate was 55%, but grade 4 neutropenia occurred in 92% and the grade 3/4 infection rate was 61%. For patients with advanced-stage disease, who are sufficiently young and fit for intensive therapy, SMILE has emerged as preferred therapy. HDC/ASCT is also considered as consolidative therapy in advanced-stage patients. For patients with relapsed/refractory disease, PD-1 inhibition with pembrolizumab has demonstrated encouraging activity in small series and warrants further investigation.

Aggressive NK-cell leukemia

Aggressive NK-cell leukemia is a rare form of leukemia that almost always is associated with EBV infection and has a median survival of only 3 months. It is seen most often in Asians, and the median age of onset is 42 years. Typically, the bone marrow and peripheral blood are involved, in addition to the liver and spleen. Patients often have fever and constitutional symptoms and multiorgan failure with coagulopathy and hemophagocytic syndrome. It is unclear whether aggressive NK-cell leukemia represents the leukemic phase of extranodal NK-/T-cell lymphoma. There is no known curative therapy, and responses to chemotherapy are usually brief. Some encouraging results have been seen with L-asparaginase-based treatment in this disease as has been observed in patients with extranodal NK-/T-cell lymphoma, but this requires further study.

Uncommon aggressive PTCL subtypes

Subcutaneous panniculitis-like T-cell lymphoma. Subcutaneous panniculitis-like T-cell lymphoma (SCPTCL) is an uncommon PTCL subtype that preferentially infiltrates the subcutaneous tissue. It has been determined that tumors with the $\gamma\delta$ phenotype have a far inferior prognosis compared to those with the $\alpha\beta$ phenotype (5-year OS, 11% for $\gamma\delta$ vs 82% for $\alpha\beta$). In the WHO classification, SCPTCL is confined only to $\alpha\beta$ T cells, which usually have a CD4 $^-$ /CD8 $^+$ and CD5 $^-$ phenotype. Cases with a $\gamma\delta$ phenotype are combined in a new, rare PTCL entity termed *primary cutaneous $\gamma\delta$ T-cell lymphoma* (see section Primary cutaneous PTCL, rare aggressive subtypes) because of similar aggressive behavior. The optimal therapy for $\alpha\beta$ -type SCPTCL is unknown, with durable responses observed with both CHOP and immunosuppressive agents. Radiation therapy should be included for localized disease.

Hepatosplenic T-cell lymphoma. Hepatosplenic T-cell lymphoma is a rare PTCL subtype occurring usually in young men (median age 34 years) presenting with hepatosplenomegaly and bone-marrow involvement. Systemic “B” symptoms are common. Up to 20% of hepatosplenic T-cell lymphomas occur in the setting of immunosuppression, most commonly following solid-organ transplantation, and may occur a decade or longer after transplantation. It also has been observed in patients treated with azathioprine and the TNF α inhibitor, infliximab, which is used in Crohn’s disease. The splenic red pulp is diffusely involved, and the liver shows a sinusoidal pattern. Most tumor cells are CD3 $^+$, CD4 $^-$, and CD8 $^-$, and most are associated with isochromosome 7q. The majority of cases are of the $\gamma\delta$ TCR type; however, rare cases that are of the $\alpha\beta$ TCR type have been reported. The prognosis is extremely poor and long-term survival is rare. The optimal therapy is unknown; however, CHOP does not appear to cure this disease. High-dose cytarabine-based strategies, such as with IVAC (ifosfamide, etoposide, ara-c) have been reported to be more effective in case reports. Long-term survivors have been reported with allogeneic SCT, and referral for transplantation at diagnosis is suggested.

Enteropathy-associated T-cell lymphoma and monomorphic epitheliotrophic intestinal T-cell lymphoma. Recent findings have led to changes in the categorization of intestinal T-cell lymphomas. The two previously described variants of enteropathy-associated T-cell lymphoma (EATL) are now recognized as distinct; what was previously type II EATL is now designated as monomorphic epitheliotrophic intestinal T-cell lymphoma (MEITL). EATL is a rare, aggressive intestinal tumor, with a male predominance, that often occurs in

the setting of celiac disease and occurs typically in patients of northern European heritage. In contrast, MEITL shows no association with celiac disease and tends to occur in Asian and Hispanic populations. Both diseases commonly involve the jejunum or ileum with patients often presenting with abdominal pain; intestinal perforation can occur. The prognosis is extremely poor due to chemotherapy resistance and the difficulty of treatment delivery because of abdominal complications that can arise in the setting of malabsorption. In EATL, the neoplastic cells are typically polymorphous CD3 $^+$, CD7 $^+$, CD4 $^-$, CD8 $^{+/-}$, CD56 $^-$ $\alpha\beta$ T cells. In contrast, the neoplastic cells in MEITL are typically monomorphic CD3 $^+$, CD4 $^-$, CD8 $^+$, and CD56 $^+$ $\gamma\delta$ T cells.

The ITLP recently reported on 62 patients with intestinal T-cell lymphoma, which represented 5.4% of all lymphomas worldwide, occurring most commonly in Europe. EATL and MEITL represented 66% and 34% of the cases, respectively. The 5-year FFS was only 4% and OS was 20%, with the majority of patients treated with CHOP-type chemotherapy. Similar disappointing results are observed in other studies with CHOP-type therapy, which has prompted evaluation of HDC/ASCT (see Transplantation in PTCL below).

Primary cutaneous PTCL, rare aggressive subtypes

Primary cutaneous $\gamma\delta$ T-cell lymphoma. In the updated WHO classification, primary cutaneous $\gamma\delta$ T-cell lymphoma is now considered a distinct entity, which also includes cases previously known as SCPTCL with a $\gamma\delta$ phenotype, as described earlier. Clinically, the extremities are commonly affected, and the presentation can be variable, with patch or plaque disease or subcutaneous and deep dermal tumors that may exhibit necrosis and ulceration. The clonal T-cells have an activated $\gamma\delta$ cytotoxic phenotype and most are CD4 $^-$ /CD8 $^-$. Prognosis is poor in this disease, particularly with subcutaneous fat involvement, with a fulminant clinical course and chemoresistance.

Primary cutaneous aggressive epidermotropic CD8 $^+$ T-cell lymphoma. This provisional entity typically presents with generalized cutaneous lesions appearing as eruptive papules, nodules, and tumors with central ulceration and necrosis. Histologically, there is marked epidermotropism, and invasion into the dermis and adnexal structures is common. The tumor cells are CD3 $^+$, CD4 $^-$, CD8 $^+$, and cytotoxic marker-positive, and the clinical course is aggressive.

Transplantation in PTCL

Multiple retrospective studies have been published evaluating the impact of upfront transplantation in PTCL. Trial interpretation and comparisons are difficult for several

reasons, including the evaluation of heterogeneous patient populations, potential for selection bias, and the dearth of intention-to-treat (ITT) data. Because there are no reported prospective randomized phase 3 trials comparing HDC/ASCT with conventional-dose chemotherapy specifically for PTCL, it remains challenging to determine the relative impact of patient selection versus true differences in efficacy.

Several phase 2 prospective studies of upfront transplantation have been published and represent more homogeneous populations of treated patients. The Nordic Lymphoma Study Group completed the largest prospective phase 2 trial of upfront transplantation (NLGT-01) in 160 patients with PTCL, excluding ALK-positive ALCL. The planned treatment scheduled was CHOEP-14 for six cycles (CHOP-14 in patients >60 years old), followed by BEAM/BEAC and ASCT in responding patients. In total 160 patients represented the ITT population. Most patients had good functional status (71% with PS scores of 0 or 1), but 72% had an IPI score of >2. The CR rate pre-transplantation was 81% to transplantation, and the overall transplantation rate was 70% with a TRM of 4%. With median follow-up of 5-years, the 5-year PFS was 44% and 5-year OS was 51%. Patients with ALK-negative ALCL appeared to have a superior 5-year PFS (61%) compared with PTCL-NOS (38%), EATL (38%), or AITL (49%), but these results were not statistically significant. The 5-year OS for patients who underwent transplantation was 61% compared with 28% in those who did not. These results suggest that this approach may be appropriate in selected patients but it still represents level 2 evidence given the absence of data from a phase 3 trial.

For relapsed/refractory patients, HDC/ASCT represents the standard of care for eligible patients who have not undergone upfront transplant consolidation. In the original Parma study in which HDC/ASCT emerged as superior to second-line chemotherapy alone in relapsed aggressive NHL, immunophenotyping was not routinely performed. A subsequent report of prognostic factors did not identify a difference in outcome in B- versus T-cell lymphomas; however, the number of patients with PTCL was small. There has been no similar randomized study in PTCLs, but several retrospective studies report a salvage rate in this setting ranging from 18% to 60%. Given the overall body of evidence, ASCT frequently is offered to patients with PTCL with relapsed, chemosensitive disease.

Allogeneic SCT, with myeloablative or RIC, also has been reported to yield durable remission in many cases (3-year EFS, 23% to 64%). Evidence supporting a graft-vs-PTCL effect comes from studies with donor lymphocyte

infusions. The largest study published to date evaluated 77 previously treated patients with mainly myeloablative conditioning (74%). The 5-year PFS was 53%, but the TRM was 34% at 5 years. A phase 2 trial, evaluating RIC and allo-SCT in 17 patients, demonstrated a 3-year PFS of 64% with a TRM of 6%. Allogeneic transplantation is promising in the treatment of PTCL, but it is limited by the availability of stem-cell donors and by toxicity related to graft-versus-host disease.

Novel PTCL therapies

A number of agents are being explored in PTCL, three of which have FDA approval for use today in relapsed/refractory disease. Pralatrexate is a novel folate analogue that has enhanced uptake and cellular retention compared with MTX. Early studies suggested a sensitivity of TCLs over BCLs. The phase 2 PROPEL study evaluated pralatrexate (with vitamin B₁₂ and folate) in relapsed/refractory PTCLs and demonstrated an ORR of 29% (CR 11%), a median PFS of 3.5 months, and a median DOR of 10.5 months. The main toxicities were mucositis, thrombocytopenia, and neutropenia. These results led to FDA approval of pralatrexate in September 2009 for the treatment of relapsed/refractory PTCL. Of note, pralatrexate does not appear active in patients with AITL for whom other novel agents (HDAC inhibitors and brentuximab vedotin) are preferred.

As described previously, romidepsin is a HDAC inhibitor that has been evaluated in CTCLs and PTCLs. A phase 2B registration study was published evaluating romidepsin in 130 patients with relapsed or refractory PTCL. The ORR was 25% (CR 15%), median DOR was 17 months, and median PFS was 4 months, leading to FDA approval in 2011. Belinostat, another HDAC inhibitor, was FDA approved for relapsed/refractory PTCL in 2014 and demonstrates similar activity to romidepsin.

KEY POINTS

- BL should be treated with dose-intensive regimens which include CNS prophylaxis.
- Patients with congenital or acquired immunodeficiency have an increased risk of lymphoma and often respond poorly to therapy.
- PTCLs have an inferior outcome to DLBCL. The exceptions are ALK-positive ALCL and ALK-negative ALCL with *DUSP22-IRF4* rearrangements, which have a high cure rate with CHOP, CHOEP, or BV-CHP chemotherapy.

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23

Non-Hodgkin lymphomas

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Overview of lymphocyte development and classification of lymphoid malignancies

The lymphoid system forms the backbone of the human immune system, contributing to both the innate (nonspecific) immune response through natural killer (NK) cells and the adaptive (specific) immune response through B and T cells. Non-Hodgkin lymphomas are malignancies that arise from these cells, generally grouped as B-cell lymphomas and T-cell lymphomas. Knowledge of B- and T-cell development is important in understanding the biology and, in turn, in providing insight into the behavior of the numerous subtypes of these lymphomas that are derived from their normal B- and T-cell counterparts.

B-cell development and the biology of B-cell lymphomas

Common lymphoid progenitors in the bone marrow derived from hematopoietic stem cells are the source of B- and T-cells. Unlike T-cells, full B-cell maturation occurs in the bone marrow and begins with recombination of the *V*, *D*, and *J* gene segments of the immunoglobulin heavy chain (IgH) followed by the light-chain genes in order to generate a functional immunoglobulin that is expressed on the cell surface as B-cell receptor (BCR). The survival and maturation of B cells in the bone marrow, as well as the differentiation of mature B cells that have exited the bone marrow, are dependent on operative BCR signaling. Importantly, BCR signaling has also been found to be necessary for lymphoma development and evolution with many mature B-cell malignancies showing sensitivity to kinase inhibitors which disrupt BCR signaling.

Collectively, the primary function of B cells is to generate a vast diversity of immunoglobulins. Generating this diversity begins with the combinatorial diversity produced from random *V*, *D*, and *J* rearrangements. Combinatorial diversity is amplified by junctional diversity produced by the action of terminal deoxynucleotidyl transferase (TdT) where nucleotides are randomly added or deleted at the sites of *V*, *D*, and *J* fusion. Successful rearrangement of the heavy and light immunoglobulin chains (kappa or lambda) results in expression of functional IgM and IgD on the surface of mature B cells that exit the marrow. These mature, but antigen-naïve, B cells then gain additional diversity when exposed to antigens in

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Off-label drug use: Lenalidomide in follicular lymphoma; rituximab in hairy cell leukemia; bendamustine, brentuximab vedotin, gemcitabine, ibrutinib, lenalidomide and oxaliplatin in DLBCL; ibrutinib, lenalidomide, temozolamide and thioguanine in PCNSL; pembrolizumab in PMBCL; alemtuzumab, gemcitabine, lenalidomide and liposomal doxorubicin in PTCL; mogamulizumab in ATLL; crizotinib in ALK+ALCL.

the germinal centers of secondary lymphoid organs, such as lymph nodes, mucosa-associated lymphoid tissue, or the spleen. Here, somatic hypermutation occurs in the V genes of the heavy and light chains, fine-tuning their affinity to their cognate antigens. B cells expressing immunoglobulin with just the right amount of antigen affinity, differentiate to memory B cells and plasma cells while all the others undergo apoptosis. Finally, class switching occurs in the germinal center and involves changing the heavy chain that is expressed to produce IgG, IgA, or IgE.

The classification of B-cell lymphomas is based, in part, on the resemblance of a given lymphoma subtype to a particular stage in B-cell development and differentiation which reflects their origin and informs their biology (Figure 23-1). Distinct stages of B-cell development and differentiation are characterized by cytologic features, ex-

pression patterns of differentiation markers, and the B-cell antigen receptor (BCR). These characteristics form the basis of pathologic diagnosis of lymphoid neoplasms. For example, B-lymphoblastic leukemia/lymphoma arises from an immature B cell (Figure 23-1) and, accordingly, diagnosis requires the identification of immature B cells that have morphologic characteristics of blasts; coexpress B-cell markers, such as CD19, with markers of immaturity, such as TdT and CD10; and do not express BCRs on their surface. Likewise, follicular lymphoma (FL) arises from a germinal-center B cell (Figure 23-1) and has morphologic characteristics of nodular growth, resembling B-cell follicles, while expressing the germinal-center marker CD10 with surface IgM, IgD, IgG, or IgA.

The transformation of normal B cells into their malignant counterparts is closely linked to the essential role of B

Figure 23-1 Schematic representation of B-cell differentiation (WHO 2017). CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; GC, germinal center; MALT, mucosa-associated lymphoid tissue. Reproduced with permission from Harald Stein.

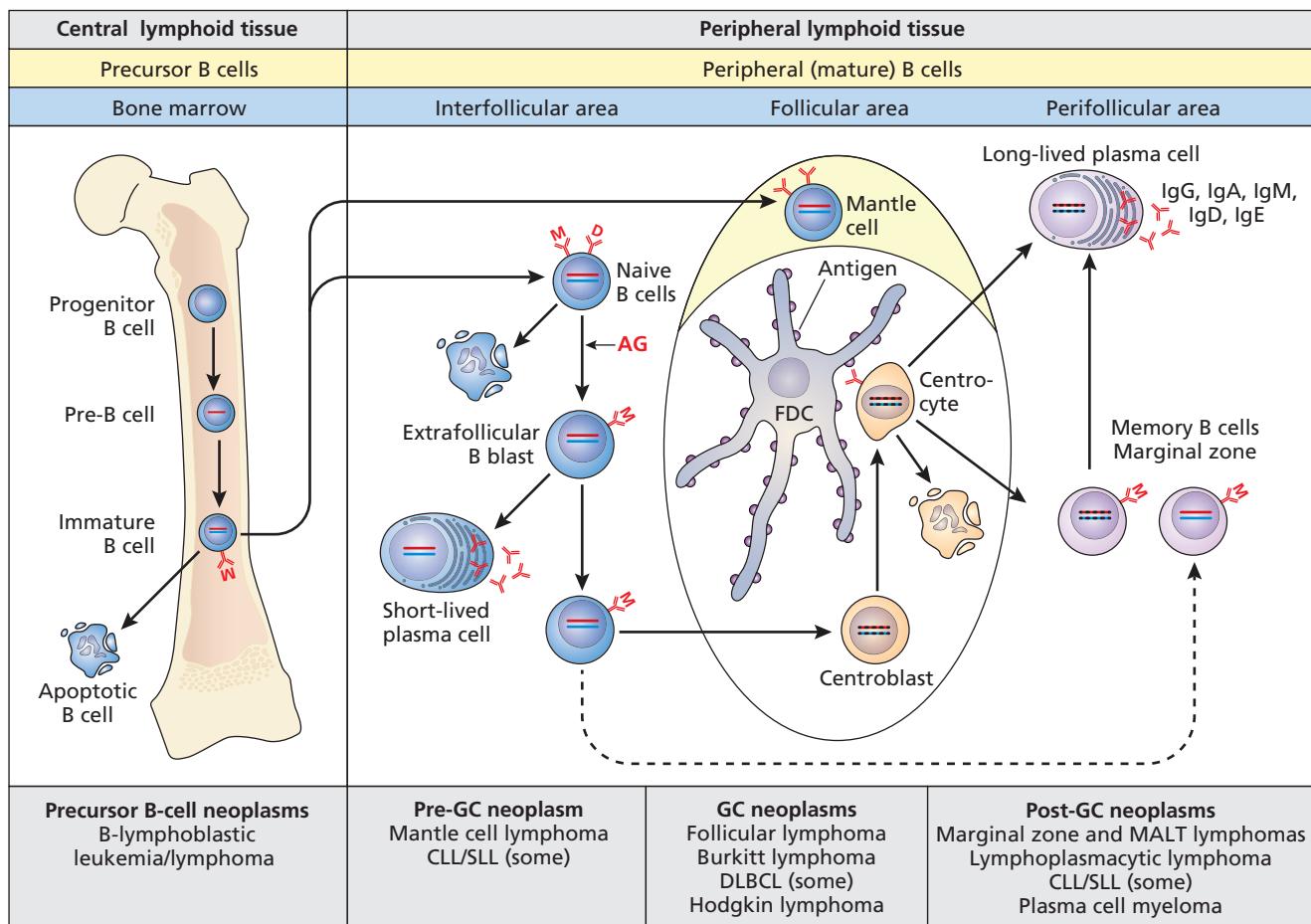


Table 23-1 Risk factors in the development of non-Hodgkin lymphoma

Viral infection	EBV, HTLV-1, HHV-8, hepatitis C virus
Bacterial infection	<i>Helicobacter pylori</i>
	<i>Chlamydophila psittaci</i>
Impaired/altered immunity	Ataxia-telangiectasia
Congenital disorders	Wiskott-Aldrich syndrome
	X-linked lymphoproliferative syndrome
	Severe combined immunodeficiency
	Other immunodeficiency states
Acquired conditions of immunodeficiency	HIV infection
	Organ or stem cell transplantation
	Aging
	Chronic immunosuppressive medications
Autoimmune and rheumatologic disease	Rheumatoid arthritis
	Systemic lupus erythematosus
	Sjögren syndrome
	Celiac disease
Environmental or occupational	Herbicides
	Pesticides

cells to generate immunological diversity, and thus, specific immunity. Conditions under which malignant transformation is fostered include viral infection, chronic bacterial infection, immune deficiency, autoimmune disease, and exposure to toxins (Table 23-1). Given the degree to which the immunoglobulin genes of B cells are subjected to DNA damage in the bone marrow and germinal centers, it is not surprising that reciprocal translocations, involving an immunoglobulin gene locus and a proto-oncogene, form the hallmark of many types of B-cell lymphoma (Table 23-2).

T-cell development and biology of the T-cell lymphomas

In contrast to B-cell development, T-cell progenitors derived from common lymphoid progenitors exit the marrow and develop in the thymus. Similar to B cells, each T cell recognizes a specific antigen, but through a T-cell receptor (TCR) rather than a BCR. Like BCRs, diversity of TCRs is generated through recombination of *V*, *D*, and *J* gene segments of the four TCR genes, *alpha* (α), *beta* (β), *gamma* (γ) and *delta* (δ). Mature T cells express $\alpha\beta$ TCR or $\gamma\delta$ TCRs. Of note, $\alpha\beta$ TCRs can recognize antigens pre-

sented only in the context of a major histocompatibility complex (MHC) while $\gamma\delta$ TCRs do not have this restriction. As such, NK cells and $\gamma\delta$ T cells do not require antigen sensitization to become active and to operate as part of our innate, rather than adaptive, immune system. Meanwhile, as developing T-cells that express $\alpha\beta$ TCR mature in the thymus, their $\alpha\beta$ TCR is complexed with surface CD3 and CD4 or CD8, which identify helper and cytotoxic T-cell subsets, respectively (Figure 23-2).

The cell-of-origin approach that was so effective for categorizing B-cell lymphomas has been more difficult to apply to T-cell lymphomas due to a combination of factors including the complexity of mature T- and NK-cell lineages, with numerous functional subsets demonstrating marked phenotypic and morphologic diversity compounded by evidence of plasticity. In addition, with the noticeable exception of anaplastic lymphoma kinase-positive (ALK-positive) anaplastic large-cell lymphoma (ALCL), few recurrent cytogenetic abnormalities have been associated with mature T-cell lymphomas and, accordingly, contribute little to their categorization. Instead, clinical features and anatomic location of the disease have played major roles in defining many of the mature T- and NK-cell neoplasms included in the World Health Organization (WHO) classification, which can be grouped according to their presentation as predominantly leukemic, extranodal, or nodal disease (Table 23-3).

Diagnostic testing in lymphoproliferative disorders

Diagnosis of lymphoproliferative disorders requires some expertise and relies on a combination of morphologic findings (peripheral blood, bone marrow, or lymph node), immunophenotyping, cytogenetics, and molecular genetics.

Morphology

Well-stained peripheral blood and bone-marrow-aspirate smears provide excellent cytologic detail, facilitating evaluation of nuclear chromatin patterns and cytoplasmic coloration as well as revealing the presence of cytoplasmic inclusions and vacuoles in lymphoid cells. The degree of nuclear chromatin condensation is helpful in differentiating lymphoid blasts, which have finely granular or “open” chromatin, from mature lymphocytes, which have more opaque and condensed chromatin. Some lymphoid malignancies, such as chronic lymphocytic leukemia (CLL), have characteristic patterns of chromatin condensation, with CLL lymphocytes typically showing a “soccer ball” nuclear pattern. Likewise, Burkitt lymphoma (BL) cells can be recognized on smear preparations by their fine granular chromatin and strikingly blue, vacuolated cytoplasm.

Table 23-2 Phenotypic markers and common chromosomal translocations in selected non-Hodgkin lymphoma subtypes

NHL	slg	CD5	CD10	CD20	Other	Cyclin D1	Cytogenetics	Oncogene	Function
CLL/SLL	Weak	+	-	Dim	CD23 ⁺ , CD200 ⁺ , FMC ⁻	-	No diagnostic abnormalities*	-	-
Follicular	++	-	+	+	BCL2 ⁺ , BCL6 ⁺	-	t(14;18)	BCL2	Antiapoptosis
Mantle cell	++	+	-	+	cyclin D1 ⁺ , CD23 ⁻ , CD200 ⁻ , FMC ⁺	+	t(11;14)	Cyclin D1	Cell cycle regulator
Marginal zone/extranodal marginal zone lymphoma	+	-	-	+	-	-	t(11;18)	AP12-MALT	Resistance to <i>Helicobacter pylori</i> treatment
Lymphoplasmacytic lymphoma	++	-	-	+	CD25 ⁺⁻ , CD38 ⁺⁻	-	-	MYD88	Proliferation
Hairy cell leukemia	++	-	-	+	CD11c ⁺ , CD25 ⁺ , CD103 ⁺ , BRAF ⁺	Weak	-	BRAF	Proliferation
DLBCL	+	Rare	+/-	+	Variable	-	t(14;18), t(3;14), t(3;v)	BCL2	Antiapoptosis
							t(8;X)	BCL6	Transcription factor
								cMYC	Proliferation
								EZH2 [‡]	Histone modifier
								MYD88 [§]	Proliferation
PMBCL	-	-	-/+	+	CD30 ⁺⁻ , CD23 ⁺⁻ , PD-L1 ⁺⁻	-	t(16;X) [†]	CIITA	MHC class II transactivator
Burkitt lymphoma	+	-	+	+	BCL6 ⁺ , MYC ⁺ , TdT ⁻ , BCL2 ⁻	-	t(8;14), t(2;8), t(8;22)	cMYC	Transcription factor
								TCF3/ID3	Transcription factor and its negative inhibitor
ALCL, ALK positive	-	-	-	-	CD30 ⁺ , CD2 ⁺⁻ , CD3 ⁺⁻ , ALK ⁺ , EMA ⁺	-	t(2;5)	ALK	Tyrosine kinase
ALCL, ALK negative	-	-	-	-	CD30 ⁺ , CD2 ⁺⁻ , CD3 ⁺⁻ , ALK ⁻ , EMA ⁻	-	t(6;7)(p25.3;q32.3)	DUSP22	Phosphatase

ALCL, anaplastic large-cell lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; PMBCL, primary mediastinal large B-cell lymphoma; MALT, mucosa-associated lymphoid tissue; slg, surface immunoglobulin; SLL, small lymphocytic lymphoma; TdT, terminal deoxynucleotidyl transferase.

*A number of prognostic cytogenetic abnormalities have been identified (see Chapter 22).

†A number of partner chromosomes described.

[‡]Exclusively in GCB-like DLBCL.

[§]Exclusively in ABC-like DLBCL.

Lymph-node biopsies and bone-marrow core biopsies lack the cytologic detail of smear preparations because tissue specimens must be fixed in formalin and dehydrated, a process that shrinks the cells and obscures cytologic detail. The benefit of tissue specimens is that they provide a glimpse of the underlying architecture, a critical component in differentiating benign from malignant lymphoid proliferations and in the classification of lymphoid malig-

nancies. Lymphoid malignancies typically obliterate and “efface” underlying normal architectural features and the pattern of malignant growth, for example, nodular versus diffuse, guides subsequent classification. These patterns can be difficult to recognize in small biopsy specimens and, accordingly, needle-core biopsies of suspected lymphoid malignancies can be extremely challenging for pathologists to interpret.

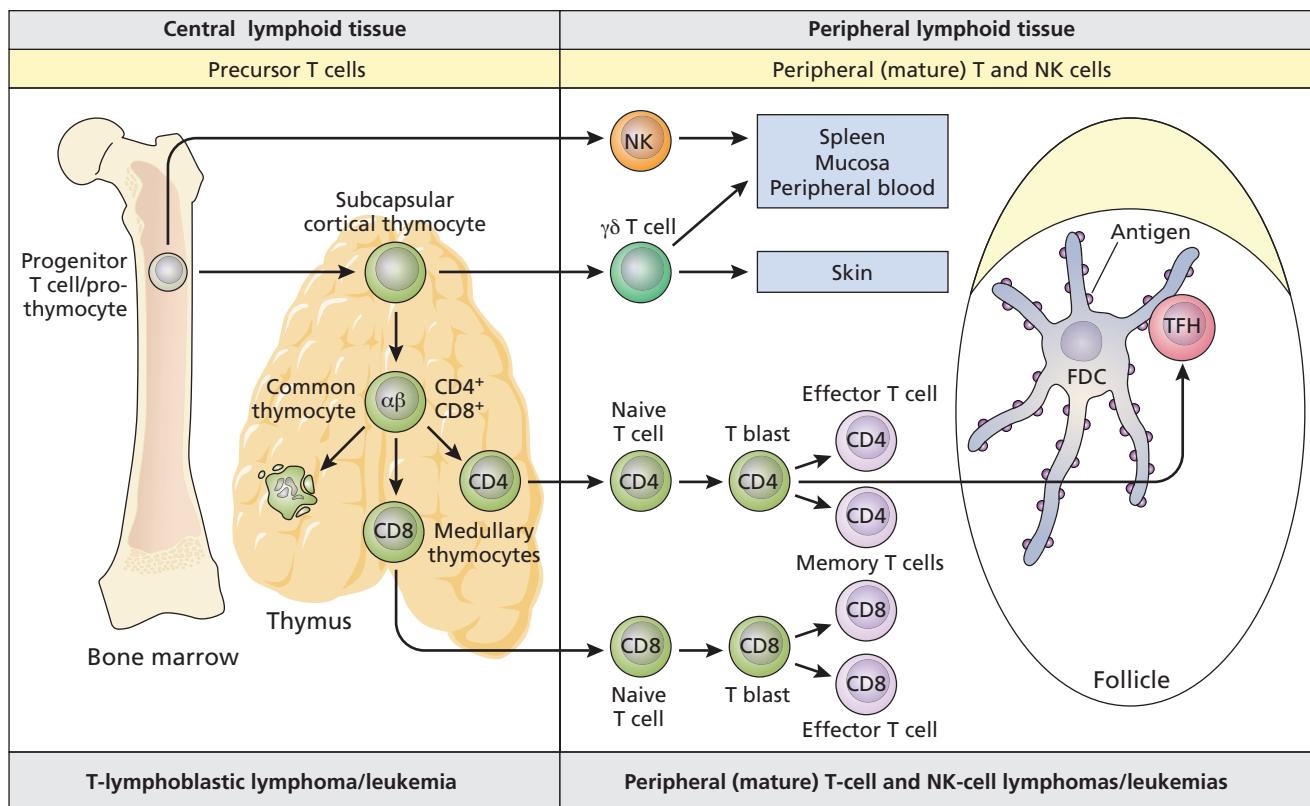


Figure 23-2 Schematic representation of T-cell differentiation (WHO 2017). FDC, follicular dendritic cells; NK, natural killer; TFH, T-helper follicular cells. Reproduced with permission from Harald Stein.

Immunophenotyping

Immunophenotyping can be performed by flow cytometry on live cells from liquid specimens or disaggregated tissue. For fixed specimens, immunophenotyping is typically performed using 3,3'-diaminobenzidine (DAB)-staining of tissue on glass slides. Immunophenotyping complements morphologic assessment by illuminating details of cell biology that would be otherwise imperceptible through the microscope. By determining cell lineage, maturation stage, and the presence of any aberrant antigen expression, immunophenotyping findings can be combined with morphologic findings to arrive at a diagnosis. For example, mantle cell lymphoma (MCL) is characterized by effacement of normal nodal architecture by small nongerminal center (CD10-negative) B cells (CD20 positive), with aberrant coexpression of CD5 (typically a T-cell marker, but expressed on a subset of B cells) and cyclin D1 (a protein that is not expressed in normal lymphocytes; its expression results from the translocation that underlies MCL). Other characteristic immunophenotypic profiles of lymphoid malignancies can be found on Table 23-2.

For B-cell malignancies, clonality can also be identified by light-chain restriction of the surface immunoglobulin. B cells normally express κ and λ light chains in a ratio of 2:1. A clonal expansion can be identified by a marked predominance of κ- or λ-expressing B cells that would not be expected in a reactive process. The immunophenotyping of T-cell neoplasms is less conclusive than for B-cell disorders because T cells lack the equivalent of light-chain restriction. Several findings can be suggestive of neoplasia, including expression of CD4 or CD8 on the majority of the T cells, lack of expression of CD4/CD8 on the majority of T cells, or coexpression of CD4 and CD8 on the majority of T cells. Often, however, molecular techniques to look at TCR gene rearrangements are necessary to differentiate reactive from clonal T-cell processes.

Molecular genetics and cytogenetics

Molecular genetic techniques can be helpful in assessing clonality when morphology and immunophenotyping are inconclusive. These techniques involve isolating the DNA from a sample and subjecting it to polymerase chain reaction (PCR) to detect rearrangements of immunoglobulin

Table 23-3 2016 World Health Organization classification of B-cell and T-cell neoplasms

B-cell neoplasms	T-cell neoplasms
Precursor B-cell neoplasms*	Precursor T-cell neoplasms*
Mature B-cell neoplasms	Mature T-cell neoplasms
B-lymphoblastic leukemia/lymphoma NOS	T-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities	
Aggressive lymphomas	Leukemic or disseminated
Diffuse large B-cell lymphoma: variants, subgroups, and subtypes/entities	T-cell large granular lymphocytic leukemia† Chronic lymphoproliferative disorders of NK cells† T-cell prolymphocytic leukemia Aggressive NK-cell leukemia Adult T-cell leukemia/lymphoma Systemic EBV-positive T-cell lymphoproliferative disorders of childhood
Diffuse large B-cell lymphoma, NOS	
Germinal center B-cell type	
Activated B-cell type	
Diffuse large B-cell lymphoma subtypes	Extranodal
T-cell/histiocyte-rich large B-cell lymphoma	Extranodal NK/T-cell lymphoma, nasal type
Primary DLBCL of the CNS	Enteropathy-type T-cell lymphoma
Primary cutaneous DLBCL, leg type	Monomorphic epitheliotrophic intestinal T-cell lymphoma
DLBCL associated with chronic inflammation	Hepatosplenic T-cell lymphoma
HHV8-positive DLBCL, NOS	Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract
EBV-positive DLBCL, NOS	Breast implant-associated anaplastic large-cell lymphoma
Other lymphomas of large B cells	Cutaneous
Primary mediastinal large B-cell lymphoma	Mycosis fungoides† Sézary syndrome†
Intravascular large B-cell lymphoma	Primary cutaneous CD30+T-cell lymphoproliferative disorder†
EBV-positive mucocutaneous ulcer	Primary cutaneous CD4+ small/medium T-cell lymphoma† Primary cutaneous acral CD8+T-cell lymphoma†
Lymphomatoid granulomatosis	Primary cutaneous anaplastic large cell lymphoma†
ALK-positive large B-cell lymphoma	Lymphomatoid papulosis
Plasmablastic lymphoma	Subcutaneous panniculitis-like T-cell lymphoma
Large B-cell lymphoma arising in HHV-8-associated multicentric Castleman disease	Primary cutaneous $\gamma\delta$ T-cell lymphoma
Primary effusion lymphoma	Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	Hydroa vacciniforme-like lymphoma
High-grade B-cell lymphoma, with <i>MYC</i> and <i>BCL2</i> and/or <i>BCL6</i> rearrangements	Nodal
High-grade B-cell lymphoma, NOS	Peripheral T-cell lymphoma, NOS
Burkitt lymphoma	Angioimmunoblastic T-cell lymphoma
Burkitt-like lymphoma with 11q aberration	Follicular T-cell lymphoma
Mantle cell lymphoma	Nodal peripheral T-cell lymphoma with TFH phenotype
In situ mantle cell neoplasia	Anaplastic large-cell lymphoma, ALK positive
	Anaplastic large-cell lymphoma, ALK negative

Table 23-3 (continued)

B-cell neoplasms	T-cell neoplasms
<i>Indolent lymphomas</i>	
Follicular lymphoma	
In situ follicular neoplasia	
Duodenal-type follicular lymphoma	
Testicular follicular lymphoma	
Pediatric-type follicular lymphoma	
Large B-cell lymphoma with <i>IRF4</i> rearrangement	
Primary cutaneous follicle center lymphoma	
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT)	
Nodal marginal zone lymphoma	
Splenic marginal zone lymphoma	
Splenic B-cell lymphoma/leukemia, unclassifiable	
Lymphoplasmacytic lymphoma	
Heavy chain disease	
Plasma cell neoplasms	
CLL/SLL	
Monoclonal B-cell lymphocytosis	
B-cell prolymphocytic leukemia	
Hairy cell leukemia	

*All precursor neoplasms are considered aggressive.

†Indolent T-cell neoplasms, all other T-cell neoplasms are considered aggressive.

CLL, chronic lymphocytic leukemia; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; NK, natural killer; SLL, small lymphocytic lymphoma.

or TCR genes. The demonstration of a dominant rearrangement of the immunoglobulin or TCR genes is indicative of a clonal process.

Chromosomal translocations are common in lymphoproliferative disorders and may contribute to the transformation process or cellular proliferation (Table 23-2). Commercial probes are available for detection of most translocations by fluorescent in situ hybridization (FISH) and can be useful markers of malignancy and for identifying specific lymphoma subtypes. Use of microarray technology has defined gene-expression profiles of various lymphoid malignancies and compared them to normal lymphoid populations. This technique has been successfully applied to a number of B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL), FL, CLL, and MCL, to identify expression patterns that correlate with patient outcome. However, technical difficulty with assessing gene-expression profiles in the clinical laboratory, especially in formalin-fixed tissues, has hampered clinical application of these findings. Despite this, pathologists and oncologists have managed to apply the

DLBCL gene-expression discoveries to the clinical realm by utilizing surrogate immunohistochemistry-based expression panels to differentiate the better-prognosis germinal-center B-cell-like DLBCL from the poor-prognosis activated B-cell-like DLBCL. More recently, next-generation-sequencing (NGS) technology has been utilized to deeply interrogate the genomes of various lymphoid malignancies. While many such studies are still ongoing, landmark discoveries of single causative mutations of *BRAFV600E* in hairy cell leukemia (HCL) and *MYD88 L265P* in Waldenström macroglobulinemia have thus far been reported (Table 23-2).

Assessment of lymphoma genetics via cell-free DNA (cfDNA) is an emerging analytic technique that has shown promise in assessing tumor kinetics, detecting occult disease, and assessing depth of response to therapy. This technique involves sequencing small fragments of cell-free DNA shed by apoptotic tumor cells into peripheral blood. Analysis of cfDNA ostensibly generates a more comprehensive assessment of tumor heterogeneity compared to tissue biopsy and facilitates serial monitoring of tumor genetics simply

by phlebotomy. For patients with B-cell lymphoma, sequencing cell-free immunoglobulin receptor (VDJ) gene sequences by NGS can identify and quantify tumor-specific rearrangements thereby facilitating assessment of tumor kinetics during therapy as well as depth of response. The kinetics and clearance of tumor cfDNA in patients with DLBCL have been associated with prolonged progression-free survival. Likewise, assessment of lymphoma-relevant mutations other than immunoglobulin receptor genes by ultra-deep sequencing of cfDNA can also be performed and clinical response in patients with DLBCL treated with R-CHOP found to be associated with clearance of cfDNA basal mutations in the peripheral blood.

Classification of non-Hodgkin lymphomas

The classification of lymphoproliferative disorders continues to evolve as our understanding of the biology of these diseases progresses. The current classification system used is the *World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues*, which was updated in 2017 (Table 23-3) and incorporates the explosion of new clinical, pathological, and genetic/molecular information that occurred since the previous 2008 publication. The B- and T-cell neoplasms are separated into precursor (lymphoblastic) neoplasms and mature B- or T-cell neoplasms. Overall, ~90% of all non-Hodgkin lymphomas (NHLs) in Western countries are of mature B-cell origin, with DLBCL and FL being the most common subtypes. In children, Hodgkin lymphoma (HL) is more predominant, and the aggressive NHLs of lymphoblastic lymphoma and BL are much more commonly encountered than are indolent neoplasms. The incidence of NHL is lower among Asian populations, in whom T-/NK-cell neoplasms are more frequent.

While the premise of the WHO classification is to separate lymphoid malignancies into distinct, nonoverlapping entities, it also recognizes that the biology of particular tumors crosses the boundaries between current categories. The classification of these gray-zone malignancies have been updated in the 2017 WHO monograph. “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma” remains unchanged, whereas “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma” has been eliminated and replaced by “high-grade B-cell lymphoma, NOS (where NOS stands for “not otherwise specified”) and “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.” Common gene expression and epigenetic profiles between primary mediastinal large B-cell lymphoma and classical Hodgkin lymphoma (cHL) indicate a true biologic gray

zone between these two entities exists. Likewise, certain cases of DLBCL have been found to have expression profiles of BL, although these cases differed clinically and genetically from classic BL and vice versa. Biologically, many of these cases may lie in the gray zone because they have rearrangements in both *cMYC* and *BCL2* or *BCL6* genes (“double-hit” lymphomas) and are more clinically aggressive than standard DLBCLs, hence their revised classification as “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.” The remaining cases that exist in the boundary between BL and DLBCL without *MYC* and *BCL2* or *BCL6* rearrangements are now classified as “high-grade B-cell lymphoma, NOS.”

For clinical purposes, the NHLs can be broadly separated into indolent or aggressive categories (Table 23-3). *Indolent lymphomas* generally are incurable with most standard therapeutic approaches and are typified by a chronic course with repeated relapses and progression with standard therapy. Some of these patients, however, survive many years with remarkably stable disease even in the absence of specific therapy. Median survival is measured in decades, and the majority of patients live a normal life expectancy compared to age-matched controls, thanks to the efficacy of modern therapy. Most, but not all, *aggressive lymphomas* are potentially curable with combination chemotherapy. Aggressive subtypes usually have a more acute presentation, often with B-symptoms, and a more rapid progression than the indolent entities. In the event of failure to achieve complete remission (CR) following treatment or with relapse after an initial therapeutic response, survival usually is measured in months rather than years. Some of these patients, however, are cured by second-line chemotherapy and stem-cell transplantation approaches as described later in this chapter.

Epidemiology, pathogenesis, and molecular characterization

Data from cancer registries show that the incidence of NHL has been increasing steadily in North America and other industrial countries with a doubling of cases between 1970 and 1990 and stabilization thereafter. In 2019, there will be an estimated 74,200 new cases of NHL, representing 4–5% of all cancer diagnoses among men and women, and 19,970 deaths. The reasons for this increasing incidence are unknown but are the subject of ongoing epidemiologic investigations. Associations have been made with occupational exposure to certain pesticides and herbicides (Table 23-1). Agricultural workers with cutaneous exposure to these agents have a 2- to 6-fold increased incidence of NHL, possibly contributing to the relatively greater frequency of lymphoma in rural vs urban populations.

Risk factors may differ between developing B- and T-cell lymphomas. A large epidemiologic study from the International Lymphoma Epidemiology Consortium (Inter-Lymph) identified eczema, T-cell activating autoimmune diseases, a family history of myeloma, and occupation as a painter as increasing the risk for T-cell lymphoma. A history of B-cell-activating autoimmune disease and hepatitis C seropositivity were associated with increased risk for certain B-cell lymphomas.

Immunosuppression associated with HIV infection or iatrogenically induced immune suppression in the organ transplantation setting is associated with an increased incidence of aggressive B-cell lymphomas, likely due to dysregulated B-cell proliferation and susceptibility to viruses, such as Epstein-Barr virus (EBV) (Table 23-1). In children, the incidence of NHL is increased in several disorders that have immunodeficiency from primary immune disorders, including ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable or severe combined immunodeficiency, and X-linked lymphoproliferative disorder.

Infection with the bacterium *Helicobacter pylori* is strongly associated with gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Table 23-1). Patients with MALT limited to the stomach often achieve CR after successful therapy to eradicate *H pylori*, indicating that the lymphoma remains dependent in part on continued antigenic drive from the microorganism. Associations have also been made between orbital infection by *Chlamydophila psittaci* and orbital adnexal MALT lymphoma, infection with *Campylobacter jejuni* and immunoproliferative small intestinal disease, and *Borrelia burgdorferi* or *Borrelia afzelii* and cutaneous MALT lymphoma. These intriguing associations need to be firmly established by additional investigation. Response to antimicrobial therapy among MALT lymphomas driven by infectious pathogens has been highly variable. The majority of gastric MALT lymphomas respond to *H. pylori* directed antibiotic treatment, while response of ocular adnexal or cutaneous MALT lymphomas to *Chlamydophila* or *Borrelia* directed therapies, respectively, has been unsuccessful overall, with some geographic variability.

Certain viral infections have been linked with specific subtypes of NHL. EBV has a clear pathogenic role in endemic, as well as in some cases of sporadic, BL and in many cases of HIV-related aggressive B-cell lymphoma and discrete subtypes of B-cell and T-cell lymphomas. EBV-positive DLBCL NOS is thought to be associated with age-related immunosuppression. EBV is strongly associated with extranodal T-/NK-cell lymphoma, nasal type, which is seen most commonly in Asia and in Central and South America. EBV is also detected in 70% to 80% of cases of angioimmunoblastic T-cell lymphoma (AITL). The gammaherpesvirus

human herpesvirus 8 (HHV-8, also called Kaposi sarcoma-associated herpesvirus [KSHV]), first described in Kaposi sarcoma but also associated with an unusual primary body cavity lymphoma (primary effusion lymphoma), is most commonly seen in patients with AIDS. HHV-8 also has been described in association with multicentric Castleman disease. The retrovirus human T-cell lymphotropic virus 1 (HTLV-1) is associated with adult T-cell leukemia/lymphoma endemic to Japan, central Africa, and the Caribbean. Chronic hepatitis C virus infection has been linked to the development of B-cell NHL, particularly marginal-zone lymphoma and DLBCL, possibly via chronic BCR stimulation through direct binding of a viral envelope protein.

Specific chromosomal translocations are strongly associated with individual subtypes of B-cell NHL (Table 23-2). The majority of these arise early in B-cell differentiation, during the process of immunoglobulin gene rearrangement, when errant fusion of immunoglobulin promoter and enhancer elements with other genes leads to dysregulated oncogene expression. Careful study of such translocations has provided important insights into pathogenic mechanisms in lymphoma. The most frequent of these translocations are: (i) t(14;18), with resultant overexpression of the anti-apoptotic gene *BCL2*, which is present in ~85% of FLs; (ii) t(11;14) with cyclin D1 overexpression, which is present in virtually all MCLs; and (iii) t(8;14), t(2;8), and t(8;22) of BL, which fuse an immunoglobulin heavy- or light-chain gene promoter to the cMYC transcription factor. *BCL6*, a chromosome-3 transcription-factor gene capable of promiscuous rearrangement with multiple translocation partners, is most commonly identified in DLBCL. The t(2;5) (p23;q35) fuses the *ALK* gene with nucleophosmin and is found in a subset of ALCL. Several other translocation partners with the *ALK* gene also have been described in this disease. This translocation and *ALK* expression are associated with a more favorable prognosis in ALCL (see also the section Peripheral T-cell lymphomas in this chapter). Among ALCL patients without an *ALK* rearrangement, DUSP22 translocations have been found in a subset of cases and predict a favorable prognosis.

Gene expression profiling has defined molecular signatures in lymphoma that have been utilized to identify prognostically significant disease subsets in DLBCL, FL, MCL, CLL, and T-cell ALCL as well as illuminating the existence of gray-zone lymphomas that lie between DLBCL and BL, as well as DLBCL and cHL. More recently, next-generation sequencing has provided some early insight into the mutational landscape of several lymphomas including the previously mentioned single causative mutations of *BRAFV600E* in HCL and *MYD88 L265P* in Waldenström macroglobulinemia. Additionally, the mutational landscape

of GCB-like DLBCL has been found to be distinct from ABC-like DLBCL, with GCB-like DLBCL harboring an activating *EZH2* mutation in a subset of cases, while ABC-like DLBCL may harbor activating *MYD88* and *CD79B* mutations. These discoveries continue to refine lymphoma classification and elucidate novel therapeutic targets.

Staging and prognostic factors

Staging procedures generally include careful physical examination for lymphadenopathy and organomegaly; computed tomography (CT) scans of the neck, chest, abdomen, and pelvis; fluorodeoxyglucose positron emission tomography (FDG-PET) imaging; and may require bone marrow biopsy. CT or magnetic resonance imaging (MRI) of the brain and evaluation of the cerebrospinal fluid are indicated in patients with BL or lymphoblastic lymphomas and should be considered in patients with DLBCL involving high-risk sites, including the paranasal sinuses or testes. The Ann Arbor staging system, identifying patients as having stage I (localized) to stage IV (extensive extranodal) disease, originally was devised for use in HL but was later adopted for use in NHL. Patients are further stratified as to the absence (A) or presence (B) of systemic symptoms, namely, fevers, drenching night sweats, or unintentional weight loss of 10% or more within 6 months of diagnosis. Several limitations become apparent when the Ann Arbor classification is applied to NHL and, as a result, a revised staging system, called the Lugano classification, was proposed in 2014 (Table 23-4). Patients with Ann Arbor stage I or II disease can be grouped and considered as having “limited stage” disease whereas patients with Ann Arbor stage III or IV disease can be grouped and considered as having “advanced stage” disease. Other recommendations from the Lugano classification include the following: (i) consider FDG-PET/CT as standard imaging for FDG avid lymphomas but employ CT for non FDG-avid histologies; (ii) reserve the suffix A or B only for HL; (iii) eliminate the X designation for bulky disease (because there is no universal definition for

bulk) and replace it with a recording of the largest nodal diameter; and (iv) eliminate the need for staging bone-marrow biopsies in aggressive NHL histologies if a PET-CT scan was used for staging.

Lymphoma staging has only limited prognostic usefulness. To more fully incorporate additional relevant prognostic features, models have been developed in multiple NHL subtypes, including DLBCL, FL, and MCL. The most widely used clinical prognostic model for stratifying patients with aggressive NHLs is the International Prognostic Index (IPI). The purpose was to identify pretreatment variables that predict relapse-free and overall survival (OS) in patients treated with doxorubicin-containing combination chemotherapy. The following five risk factors were found to be independently associated with clinical outcome and may be referred to by the mnemonic *APLES*: (i) age older than 60 years, (ii) ECOG PS > 1, (iii) elevated serum lactate dehydrogenase (LDH), (iv) number of extranodal sites of disease > 1, and (v) stage III or IV. The IPI score is derived as a simple additive score from 0–5, has been widely adopted to estimate prognosis in patients with NHL, and is useful in some of the other lymphoma subtypes. Of note, these survival estimates were established before the use of rituximab for diffuse large B-cell lymphoma.

Limited studies support that the IPI is still prognostic in the rituximab-treatment era. A revised IPI (R-IPI), based on data from the British Columbia Cancer Agency, may define new risk groups in rituximab-treated patients: very good risk (0 risk factors, 4-year progression-free survival [PFS] 90%); good risk (1, 2 risk factors, 4-year PFS 70%); and poor risk (>2 risk factors, 4-year PFS 50%). The Deutsche Studiengruppe für Hochmaligne Non-Hodgkin-Lymphome (DSHNHL) group also evaluated the usefulness of the IPI in over 1,000 patients enrolled on prospective clinical trials and found that IPI did effectively separate patients into the previously established risk categories with 3-year PFS ranging from 56% in the highest risk patients to 87% in the lowest risk (Table 23-5).

Table 23-4 Lugano staging system for NHL

Stage		Involvement	Extranodal (E) status
Lugano	Ann Arbor		
Limited	I	One node or a group of adjacent nodes	Single extranodal lesion without nodal involvement
Limited	II	Two or more lymph node regions on the same side of the diaphragm	Stage II by nodal extent with limited contiguous extranodal extension
Advanced	III	Involvement of lymph node regions on both sides of the diaphragm, nodes above the diaphragm with or spleen involvement	Stage III by nodal extent with limited contiguous extranodal extension
Advanced	IV	Additional noncontiguous extralymphatic involvement	Not applicable

Table 23-5 The IPI in DLBCL in the rituximab era

Risk factors*	3-year PFS	3-year OS
0, 1	87%	91%
2	74%	81%
3	59%	65%
4, 5	56%	59%

*IPI risk factors are age \geq 60 years, abnormal LDH, PS \geq 2, stage III or IV, and >1 extranodal sites.

Although the IPI scoring system provides useful prognostic information, there is no definitive evidence that outcome is altered by using intensive regimens in high-risk patients. Numerous studies have been reported and others are still in progress that assess the utility of the IPI and “risk-adjusted” or “risk-adapted” therapeutic strategies. These include trials of high-dose therapy (HDT) and autologous stem-cell transplantation (ASCT) for aggressive lymphoma patients with high IPI scores; however, such strategies currently are not routinely recommended because standard approaches are effective in the majority of patients, and the value of HDT has only been suggested in underpowered subset analyses of larger clinical trials showing no statistical benefit for this approach in the overall patient population (see the section “Diffuse large B-cell lymphoma” later in this chapter). The IPI is useful in comparing studies and also in the investigation of new prognostic factors to determine the independent effect on outcome.

The IPI score is predictive of survival in indolent lymphomas, namely, FL, although using the IPI, the majority of these patients fall into the low-risk or low-intermediate-risk categories. As such, a new index was developed specifically for FL, called the Follicular Lymphoma International Prognostic Index (FLIPI), in hopes of better stratifying patients (Table 23-6). This index can be remembered by the mnemonic No-LASH. The five clinical factors that are the strongest predictors of outcome in multivariate analysis were: (i) number (no.) of nodal sites of disease (>4), (ii) elevated LDH, (iii) age older than 60 years, (iv) stage III or IV disease, and (v) hemoglobin <12 g/dL. Compared with the IPI, the FLIPI provides a better distribution of patients across the risk categories of low risk (0 to 1 factor), intermediate risk (2 factors), or high risk (>2 factors). The 10-year OS rates were 71% (low risk), 51% (intermediate risk), and 36% (high risk), respectively (Table 23-6). Similarly, an international prognostic index for MCL (the Mantle Cell Lymphoma International Prognostic Index [MIPI]) also has been developed and incorporates age, performance status (PS), LDH, and white blood cell (WBC) level (Table 23-7).

Role of FDG-PET imaging

FDG-PET scanning is useful both for staging and for assessing response to lymphoma therapy and is generally recommended as part of routine staging and end-of-treatment response assessment in FDG-avid lymphomas. The 5-point scale (Deauville criteria [Table 23-8]) should be used for PET interpretation, and scores of 1 to 3 at completion of

Table 23-6 The Follicular Lymphoma International Prognostic Index (FLIPI)

Risk model and group	No. of factors	Distribution of cases (%)	5-year OS (%)	10-year OS (%)
FLIPI*				
Low	0–1	36	91	71
Intermediate	2	37	78	51
High	³ 3	27	53	36

*FLIPI risk factors: No-Lash, number of nodal sites of disease (>4); elevated LDH, age >60 years, stage III or IV disease, and hemoglobin ≤ 12 g/L.

Table 23-7 The Mantle Cell Lymphoma International Prognostic Index (MIPI)

Points	Age, years	ECOG PS	LDH/ULN	WBC, cells/mm ³
0	<50	0–1	≤ 0.67	$<6,700$
1	50–59	—	0.67–0.99	6,700–9,999
2	60–69	2–4	1.00–1.49	10,000–14,999
3	³ 70	—	≥ 1.50	$\geq 5,000$

MIPI risk factors are age, PS, LDH, WBC level.

Formula for MIPI: $[0.03535 \times \text{age (years)}] + 0.6978$ (if ECOG >1) $+ [1.367 \times \log_{10}(\text{LDH}/\text{ULN})] + [\log_{10}(\text{WBC count})]$.

Simplified MIPI: low risk, 0–3 points; intermediate risk, 4–5 points; high risk, 6–11 points.

ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; PS, performance status; ULN, upper limit of normal; WBC, white blood cell.

Table 23-8 Deauville 5-point scale for PET interpretation in lymphoma

Score	Visual description
1	No uptake
2	Uptake \leq mediastinum
3	Uptake $>$ mediastinum but less than liver
4	Uptake moderately higher than liver
5	Uptake markedly higher than liver

therapy are considered consistent with complete remission, regardless of the size of any residual masses. Some studies indicate that interim PET scanning, performed mid-treatment, can identify patients at higher risk for treatment failure; however, it is unknown whether therapy should be altered based upon the results of a mid-treatment PET scan. False-positive results can occur in the setting of inflammation, granulomatous disease, and infection, and a biopsy should be performed in a PET-positive patient in remission by CT scan if high-dose chemotherapy and stem-cell transplantation (HDC/SCT) are under consideration.

Patient management and follow-up

With over 60 lymphoma subtypes, detailed management guidelines for each subtype and disease stage are beyond the scope of this chapter. The reader is encouraged to refer to the NCCN guidelines at <http://www.nccn.org/> which is an outstanding resource for the treating clinician.

Patient surveillance following treatment of lymphoma should address both long-term complications of therapy and disease recurrence. Long-term effects of therapy depend on the type of treatment and whether radiotherapy was also administered. For example, radiotherapy to the head and neck region leads to decreased salivation with dental caries, and if the thyroid is included in the radiation field, a large proportion of patients eventually will become hypothyroid. Women who have had mantle radiation should receive a mammogram beginning 10 years after radiation or at age 40 years, whichever comes first. In younger women, MRI breast imaging also can be considered, given the reduced sensitivity of mammography in this population.

Long-term survivors are at risk of second malignancies, which are dependent on the treatment administered. For example, radiated patients are at risk for carcinomas and sarcomas in the radiated field, while those who have had alkylating agents are at risk for therapy-related myelodysplastic syndrome or acute myeloid leukemia. Once primary therapy has been completed and remission is documented, patients typically are followed every 3 months for the first 2 years, then every 6 months until 5 years, and then annually thereafter.

Most recurrences of aggressive lymphoma occur in the first 2 years after treatment, although late relapses beyond 5 years do occur in a minority of patients. Patients with indolent lymphoma have a lifelong risk of relapse and typically are seen every 3 months for the first 2 years and then every 6 to 12 months indefinitely. There is no evidence that routine CT or PET imaging affects outcome of patients, and newer guidelines recommend minimizing surveillance imaging in indolent lymphomas and discourage any minimal use of surveillance imaging in aggressive lymphoma.

KEY POINTS

- NHLs are biologically and clinically heterogeneous; accurate diagnosis by a hematopathologist using the WHO classification is essential for optimal management.
- The majority of NHLs are of B-cell origin and are categorized broadly as indolent vs aggressive subtypes.
- The incidence of NHL is increasing in Western countries.
- Specific chromosomal translocations are associated with specific subtypes of lymphoma and are pathogenetically involved in malignant transformation and progression.
- The IPI score provides important prognostic information for outcome and survival in aggressive lymphomas. The FLIPI has been developed specifically for FL.

Indolent B-cell NHL

The indolent B-cell lymphomas include the histologies shown in Table 23-3, and the most commonly encountered subtype is FL, which accounts for 20% to 30% of all lymphomas. Other subtypes include marginal-zone lymphomas (nodal, splenic, and extranodal [MALT] types) and lymphoplasmacytic lymphoma. This category also includes CLL/SLL, which is discussed in Chapter 24.

CLINICAL CASE

A 53-year-old man is diagnosed with stage IV FL after noticing a lump on his neck while shaving. A biopsy reveals a lymph node with enlarged, closely packed follicles with distorted architecture. Inside the follicles are small lymphocytes with irregular nuclei. The cells stain positive for CD20, CD10, and BCL2. The staging evaluation reveals widespread lymphadenopathy, involving five nodal groups, with the largest node measuring just over 3 cm. The hemoglobin and LDH are normal. He has no disease-related symptoms and his Eastern Cooperative Oncology Group (ECOG) PS is 0. The FLIPI score is 2, and he has a low tumor burden by Groupe d'Etude des Lymphomes Folliculaires (GELF) criteria.

Follicular lymphoma

FL is the prototypical and most common indolent lymphoma, with about 15,000 new cases diagnosed each year in the United States. Although incurable, the prognosis is quite good and has substantially improved in the modern era with the majority of patients now predicted to have a normal life expectancy compared to age-matched controls.

FLs are derived from germinal-center B cells and are graded based on the number of centroblasts per high-power field: grade 1-2 (0-15), and grade 3 (>15). Grade 3 is further classified into grade 3A (centrocytes present) and grade 3B (solid sheets of centroblasts). Grade 1-2 constitutes the typical low-grade follicular lymphoma, while grade 3 FL is relatively uncommon (<20% of all FLs); the natural history of this entity is less clear but may behave more aggressively. Most contemporary clinical trials will allow grade 3A to be included with grade 1-2 cases, whereas grade 3B is excluded and managed akin to DLBCL. Immunophenotypically, FL cells are CD20⁺, CD10⁺, BCL6⁺, BCL2⁺, and CD5⁻. Up to 90% of cases have a t(14;18) with a higher frequency observed in grade 1-2 FLs.

The 2016 WHO classification has identified several variants of FL. These include in-situ follicular neoplasia, duodenal-type follicular lymphoma, and testicular follicular lymphoma; alongside three separately classified indolent B-cell lymphomas of follicle-center origin, primary cutaneous follicle-center lymphoma, pediatric-type follicular lymphoma and large B-cell lymphoma with *IRF4* rearrangement. “In situ follicular neoplasia” replaced the previous diagnosis of “in situ follicular lymphoma,” consistent with growing conservatism in diagnosis of lymphoid neoplasia with a low rate of progression. Both duodenal-type and testicular follicular lymphomas are localized, biologically distinct, extranodal variants of FL that have excellent long-term outcomes with watch-and-wait approaches after surgical excision.

Primary cutaneous follicle-center lymphoma should be distinguished from FL. It is derived from follicle-center cells and can have a follicular, follicular and diffuse, or diffuse growth pattern. Unlike nodal FL, the neoplastic cells are usually BCL-2 negative and typically occur as solitary or localized skin lesions on the scalp, forehead, or trunk; only 15% present with multi-focal lesions. The clinical course is usually very indolent and can be managed with low-dose radiation and other site-directed approaches.

Likewise, pediatric-type follicular lymphoma and large B-cell lymphoma with *IRF4* rearrangement are distinguished from FL in the 2016 WHO. As the name suggests, pediatric-type FL typically occurs in children and young adults and is a nodal disease characterized by large

expansile highly proliferative follicles comprised of blas-toid cells that lack the typical t(14;18) translocation and are BCL2 negative. Despite the aggressive cytologic features, the prognosis is excellent with nearly all cases presenting with localized disease that may not require treatment other than excision. Large B-cell lymphoma with *IRF4* rearrangement also typically occurs in children and young adults, involving Waldeyer ring or cervical lymph nodes, with a follicular or diffuse pattern of intermediate-to-large follicle-center B cells that aberrantly coexpress the post-germinal-center protein IRF4/MUM1. In contrast to pediatric-type follicular lymphoma, patients with large B-cell lymphoma with *IRF4* rearrangement typically require combination immunochemotherapy with or without local radiation.

Management of localized follicular lymphoma

Limited-stage (Ann Arbor I or II) FL is relatively uncommon and, as a result, there are no randomized studies indicating the optimal management strategy. Rather, most of the data are observational. Older studies suggested a proportion of patients might be cured with external beam radiation. MacManus and Hoppe (1996) found that ~40% of limited-stage patients with FL remained disease-free at 10 years after radiation treatment; late relapses beyond 10 years were unusual. Other studies also reported a 10-year disease-free survival (DFS) rate of ~40% to 50%, suggesting that cure is possible with this approach in a proportion of patients. Given the excellent long-term outcomes for patients with localized FL, there is concern for late-onset radiation-induced complications, including second primary cancers. Recent data indicate that radiation fields can be reduced without adversely impacting disease control. As a result, contemporary strategies tend to utilize an involved-site approach. Studies evaluating chemotherapy plus radiation (combined modality therapy [CMT]) have demonstrated improved PFS without an obvious effect on OS. Therefore, the CMT approach is likely best reserved for the rare patient who presents with bulky (node >7 cm) limited-stage FL. Finally, an alternative management strategy for this patient population is surveillance alone. A Stanford report of stage I and II patients, who received no initial therapy, showed that more than half of the 43 patients did not require therapy at a median of 6 years and that 85% of patients were alive at 10 years. A report from a large observational database found that the following treatment approaches were utilized for 471 stage I FL patients: rituximab combined with chemotherapy 28%, radiation therapy (XRT) 27%, observation 17%, CMT 13%, rituximab 12%, and other 3%. Approaches utilizing systemic therapy produced better PFS outcomes than XRT alone, but there were no OS differences between any of

the approaches; therefore optimal management should be personalized for the patient.

Approach to patients with advanced-stage follicular lymphoma

Patients with advanced-stage FL are considered incurable with standard chemotherapy. The disease generally is responsive to treatment, however, and there are numerous effective treatment options. As a result, the prognosis is excellent relative to other cancers. A typical patient undergoes a number of different treatments, often separated by several years, and the goal of management is to achieve a normal life expectancy. Advanced-stage FL can be thought of as a chronic disease that requires long-term management, and the management is largely a matter of determining how to sequence the different therapies.

The approach to a newly diagnosed patient needs to be individualized, factoring in the presence or absence of symptoms, tumor burden, patient age and comorbidities, and goals of therapy. A 2×2 table can be constructed to help with the initial approach of separating patients by symptoms and tumor burden (Table 23-9). Using this approach, four patient categories are generated: (i) asymptomatic, low tumor burden; (ii) asymptomatic, high tumor burden; (iii) symptomatic, low tumor burden; and (iv) symptomatic, high tumor burden. Patients with asymptomatic, low tumor burden should be followed with surveillance alone. Patients with asymptomatic, high-tumor-burden FL should generally start therapy soon after diagnosis, although selected patients may be observed initially, such as the very elderly or those who just meet the high-tumor-burden criteria (eg, three nodes in the 3- to 4-cm range). Patients with symptomatic, low-tumor-burden disease do benefit from therapy, often with mild treatment approaches including rituximab alone or low-dose radiation. From a decision-making standpoint, patients with symptomatic, high-tumor-burden FL are the most straightforward. They require treat-

ment, typically with chemoimmunotherapy, although there is little consensus on which specific chemoimmunotherapy regimen is best.

Management of asymptomatic, low-tumor-burden follicular lymphoma

Asymptomatic patients may be candidates for a strategy of surveillance alone. To determine whether observation is an option, one should assess the tumor burden. The GELF criteria (Table 23-10) are the most commonly used criteria to assess tumor burden and to assess eligibility for clinical trials. The surveillance strategy was first advocated at Stanford University when two retrospective studies suggested no detriment in patient outcome. Three randomized clinical trials in the pre-rituximab era later confirmed that low-tumor-burden FL patients assigned to surveillance alone experienced the same OS compared with patients assigned immediately to treatment. The median time to first chemotherapy in all studies was 2.3–3 years. More recently, a randomized trial compared surveillance alone with single-agent rituximab in patients with previously untreated, asymptomatic, low-tumor-burden FL. Patients were assigned to surveillance (arm A), rituximab at 4 weekly doses (arm B), or rituximab at 4 weekly doses plus a single dose every 2 months for 2 years (arm C). A significant prolongation in PFS and prolongation in the time to first chemotherapy was observed for the patients randomized to rituximab; however, there was no difference in OS at 3 years (95% in all arms), consistent with randomized trials in the pre-rituximab era. The study also evaluated quality of life (QOL). Given that these patients are symptom free, the main QOL issues tend to be anxiety, depression, and adjustment to illness. The study found that anxiety and depression were more common in patients with low-tumor-burden FL than in the general population but were still relatively infrequent at 13% and 3%, respectively. Patients in all treatment arms adapted to their illness over time. The

Table 23-9 Algorithm for the approach to the newly diagnosed FL patient

	Low tumor burden	High tumor burden
Symptoms absent	Surveillance	R-chemotherapy +/-MR
Symptoms present	Single-agent rituximab, low dose radiation to single symptomatic site of disease, or R-chemotherapy	or O-chemotherapy +/-MR or rituximab monotherapy or surveillance in older/less fit patients

R, rituximab; MR, maintenance rituximab; O, obinutuzumab; MO, maintenance obinutuzumab.

Table 23-10 GELF criteria for high tumor burden

Any nodal or extranodal mass >7 cm
Three or more nodal sites with diameter of >3 cm
Elevated LDH
Hb <10 g/dL, ANC <1.5 × 10 ⁹ /L, Plts <100 × 10 ⁹
Spleen >16 cm by CT scan
Risk or organ compression or compromise
Significant serous effusions

Meeting any one criterion qualifies as high tumor burden. All must be absent to qualify as low tumor burden.

ANC, absolute neutrophil count; GELF, Groupe d'Etude des Lymphomes Folliculaires; Hb, hemoglobin; LDH, lactate dehydrogenase; Plts, platelets.

patients identified as “anxious” adapted more readily when assigned to rituximab treatments. It is reasonable to conclude that, given no OS difference observed to date, surveillance remains the appropriate standard for the asymptomatic, low-tumor-burden FL population, though rituximab monotherapy can be considered in selected patients.

If administering single-agent rituximab to a patient with low-tumor-burden FL, should one utilize a maintenance strategy or simply retreat at progression? This dosing question was addressed in the RESORT study. After induction therapy with single-agent rituximab, patients with low-tumor-burden indolent B-cell NHL were randomized to receive maintenance rituximab once every 3 months until treatment failure or to be periodically retreated with rituximab (retreated with 4 weekly doses at each progression) until treatment failure. The trial revealed no difference in the time-to-treatment failure between the two dosing strategies. Patients on the maintenance arm, however, utilized four times as much rituximab. There was no difference in quality of life, depression, or anxiety between the two strategies. Based on these results, a retreatment strategy is preferred if opting for single-agent rituximab in this patient population.

Therapy for symptomatic and/or high-tumor-burden follicular lymphoma

Treatment is indicated for FL when patients develop adverse symptoms related to their disease, or develop bulky disease which is at high risk for causing symptoms or obstruction in the near future. The addition of rituximab to conventional chemotherapy has improved outcomes in FL, including response rates, PFS, event-free survival (EFS), and OS. Table 23-11 summarizes major studies combining rituximab with chemotherapy.

Clearly, rituximab added to chemotherapy is a therapeutic advance in FL, though the optimal chemotherapy backbone remains unsettled. Data generated prior to the introduction of bendamustine in the US indicated the most commonly used regimens in the United States were R-CHOP (rituximab, cyclophosphamide, vincristine,

prednisone) (60%), R-CVP (rituximab, cyclophosphamide, prednisone) (27%), and R- fludarabine-based (13%). A randomized comparison of these regimens indicated R-CHOP had the best risk-benefit profile because it was more active than R-CVP and less toxic than R-FM. Subsequently, however, bendamustine, an alkylating agent with nucleoside-analogue properties, gained widespread adoption as the chemotherapy platform of choice in FL. A phase 3 trial comparing bendamustine plus rituximab (BR) to R-CHOP demonstrated better efficacy and reduced toxicity with BR. In this multicenter phase 3 study, 549 patients with high-tumor-burden indolent NHL and MCL (median age 64 years) were randomized to receive bendamustine 90 mg/m² on days 1 and 2, with rituximab 375 mg/m² on day 1, every 28 days (the BR group) or to receive standard R-CHOP chemotherapy every 21 days (the R-CHOP group). The overall response rates (ORRs) were similar in the BR and R-CHOP groups (92.7% vs 91.3%, respectively), but the CR rate was significantly higher in the BR group (39.8%) compared with the R-CHOP group (30.0%) ($P=.03$). When evaluating just the FL patients, with a median follow-up of 45 months, the median PFS was significantly longer in the BR group compared with R-CHOP group (median PFS, not reached vs 40.9 months, $P=.007$). OS did not differ between both groups. There was less hematologic toxicity, alopecia, infections, peripheral neuropathy, and stomatitis with BR. Drug-associated erythematous skin reactions were seen more frequently in the BR group. These data suggest that BR is a better option for untreated high-tumor-burden FL.

A confirmatory randomized phase 3 trial (BRIGHT study) was conducted in North America. Previously untreated indolent NHL patients with high tumor burden were randomized to BR or R-CHOP/R-CVP. Control arm patients were identified as R-CHOP or R-CVP candidates prior to randomization. The primary endpoint was to show noninferiority of BR in the CR rate. Seventy percent of the 447 enrolled patients had FL, and, in these

Table 23-11 Randomized trials of chemotherapy versus R-chemotherapy in high tumor burden, advanced-stage follicular lymphoma

Study	Treatment	N	Median follow-up	ORR	Time to event	OS
Hiddemann et al, <i>Blood</i> 2005	R-CHOP vs CHOP	223 vs 205	1.5 years	96% vs 90%	88% vs 70% (2-year DOR)	95% vs 90% (2-year OS)
Marcus et al, <i>J Clin Oncol.</i> 2008	R-CVP vs CVP	162 vs 159	4.5 years	81% vs 57%	38 months vs 14 months (median DOR)	83% vs 77% (4-year OS)

CVP, cyclophosphamide, vincristine, prednisone; DOR, duration of response; DFS, disease-free survival; EFS, event-free survival; R-CVP, rituximab, cyclophosphamide, vincristine, prednisone.

patients, BR therapy was found to be noninferior to the R-CHOP/R-CVP control arm for CR rate (30% vs 25%) and overall response rate (99% vs 94%). Time-to-event data were not reported. Side-effect profiles were distinct, with more GI toxicity and rash with BR and more neuropathy and alopecia with R-CHOP/R-CVP. Although, the BRIGHT data do not exactly replicate the StIL data for BR, they do suggest that BR remains a very reasonable alternative to R-CHOP or R-CVP in FL.

The question of whether to administer maintenance rituximab after frontline R-chemotherapy was addressed in the phase 3 PRIMA trial. The study evaluated the efficacy and safety profile of maintenance rituximab in newly diagnosed FL patients who responded to initial treatment with rituximab plus chemotherapy. Chemotherapy backbone was selected by treating center: R-CHOP (75%), R-CVP (22%), or R-FCM (3%). Patients were randomized to observation or to a single dose of rituximab every 2 months for 2 years. At a median follow-up of 36 months from randomization, the 2-year PFS in the maintenance rituximab arm was 75% versus 58% in the observation arm ($P<0.0001$). The beneficial effect of maintenance rituximab was seen irrespective of the induction chemotherapy backbone and in both CR and partial remission (PR) patients. Grade 3–4 adverse events were slightly higher in the maintenance rituximab arm (24% vs 17%). No difference in OS was observed. Given the lack of OS benefit, the decision regarding the use of maintenance rituximab can be individualized. Rituximab administration does carry a low risk for neutropenia and low-grade infections, rarely, more serious toxicities, such as progressive multifocal leukoencephalopathy. As maintenance, rituximab generally is well tolerated and it has become a commonly utilized strategy in the United States.

More recently, the next-generation anti-CD20 monoclonal antibody obinutuzumab was compared with rituximab when combined with initial chemotherapy followed by maintenance in high-tumor-burden patients with follicular lymphoma. A total of 1,202 patients were randomized to obinutuzumab-chemo followed by obinutuzumab maintenance, vs rituximab-chemo followed by rituximab maintenance. Choice of chemotherapy backbone was at the discretion of participating centers and included bendamustine (57%), CHOP (32%), and CVP (10%). Dosing was different for the two antibodies, with obinutuzumab patients receiving more monoclonal antibody. Rituximab was administered at the standard dose of 375 mg/m^2 on day 1 of each chemoimmunotherapy cycle, while obinutuzumab was dosed at 1,000 mg on days 1, 8, and 15 during cycle 1, and then on day 1 of subsequent chemoimmunotherapy cycles. Maintenance was administered at the

same dose of the respective antibodies every 2 months for up to 2 years. The study showed no difference in overall or complete response rate between the two antibody strategies at the end of induction. During the maintenance period, however, a PFS benefit emerged in favor of obinutuzumab therapy with 3-year PFS of 80.0% vs 73.3%, and a hazard ratio of 0.66 (95% confidence interval, 0.51–0.85, $P=.0001$). There was no difference in OS, and toxicity was increased in the obinutuzumab arm with higher rates of neutropenia and infusion-related reactions. Based on these data, obinutuzumab-based chemoimmunotherapy plus maintenance is now an FDA approved initial treatment option for high-tumor-burden FL patients, but, in the absence of an OS benefit and with increased toxicity, rituximab-based therapy also continues to be an acceptable alternative. Notably, all patients in this trial received induction therapy followed by maintenance therapy, so, for patients planned for treatment with induction therapy alone without maintenance, rituximab-based treatment remains the most appropriate therapy.

Therapy for relapsed and refractory follicular lymphoma

Multiple options exist for the treatment of patients who have progressed after first-line therapy, and the decision of which therapy to use depends on a number of factors, including the prior treatment utilized, duration of prior response, patient age, comorbid illnesses, and goals of therapy. Options range from low-risk strategies, such as single-agent rituximab, to higher intensity strategies, such as autologous or allogeneic stem-cell transplantation, with many options in between. Population-based data and a report from the national LymphoCare study both show that patients who relapse within 2 years of initial chemoimmunotherapy have a significantly inferior overall survival compared to patients with longer initial remissions. Among the 80% of patients who enjoy an initial remission longer than 2 years, their predicted life expectancy is no different when compared to age-matched controls without lymphoma.

These high-risk patients with early progression of disease constitute an unmet medical need within relapsed FL and warrant evaluation in clinical trials of novel treatment approaches.

Bendamustine is approved in the United States for use in patients with rituximab-refractory indolent B-cell lymphoma. A pivotal trial in 100 patients reported an objective response rate (ORR) of 75% with a median PFS of 9.3 months. A subsequent randomized trial compared bendamustine alone to bendamustine combined with obinutuzumab, followed by obinutuzumab mainte-

nance, in rituximab-refractory FL. Patients treated with obinutuzumab-bendamustine demonstrated an improved PFS and OS compared to bendamustine alone, making this a preferred option in rituximab-refractory patients. An important caveat is that patients in this trial were bendamustine naïve, so this strategy has not proven beneficial in patients already treated with bendamustine therapy in the frontline setting.

Novel targeted therapies are playing an increasing role in the management of relapsed and refractory follicular lymphoma. The oral immunomodulating agent lenalidomide was evaluated as monotherapy or in combination with rituximab in a randomized trial for rituximab-sensitive FL, with lenalidomide -rituximab demonstrating an ORR and CRR of 76% and 39%, respectively, and a median time to progression of 2 years. Lenalidomide can now be considered an effective therapy for relapsed FL and is currently under evaluation as frontline therapy. Two targeted inhibitors of PI3K delta are also now available for patients with FL who have relapsed after at least two prior lines of therapy. The oral PI3K delta inhibitor idelalisib was evaluated in a phase 2 study of 125 patients with indolent NHL who were considered refractory to both rituximab and an alkylating agent. Idelalisib was administered at a dose of 150 mg BID until PD or patient withdrawal. The response rate was 57% with a median duration of 12.5 months. Grade 3 or higher toxicities included neutropenia (27%), transaminase elevations (13%), diarrhea (13%), and pneumonia (7%). Copanlisib, an intravenous inhibitor of PI3K delta and alpha, was also FDA-approved for this indication based on a phase 2 study in 142 patients with relapsed or refractory indolent lymphoma which had relapsed after at least 2 prior therapies. Copanlisib was administered intravenously on days 1, 8, and 15 of a 28-day cycle and continued until progression or intolerance. The ORR was 59% including 12% CRs and a median duration of response of 22.6 months. The most common grade 3–4 toxicities included hyperglycemia (41%), hypertension (24%), neutropenia (24%), and pneumonia (15%). More recently, the oral PI3K inhibitor duvelisib also demonstrated significant clinical activity in multiply relapsed FL with a similar safety profile to the other agents. All three PI3K inhibitors are FDA-approved for FL patients who have relapsed after at least 2 prior lines of therapy and represent effective treatment options in multiply relapsed/refractory disease, but their use in therapy requires counseling and monitoring for their unique toxicity profiles.

Radioimmunotherapy (RIT) is also an option for patients with indolent B-cell NHL if the bone marrow is minimally involved and the disease is not bulky. With Y⁹⁰

ibritumomab tiuxetan, response rates are ~70% and response duration is, on average, 11–15 months. Single-agent rituximab can be used in relapsed lymphoma, although now that most patients have received it with their primary therapy, and often as maintenance therapy, more and more patients are becoming rituximab-refractory. For patients who are still rituximab-sensitive, single-agent rituximab is an attractive option for elderly or unfit patients.

Stem-cell transplantation

HDC with autologous stem-cell transplantation (ASCT) and allogeneic stem-cell transplantation (allo SCT) are both useful strategies in the management of selected patients with FL, particularly for younger patients with high-risk features, such as a brief remission after initial therapy. A review of 904 patients in the International Bone Marrow Transplant Registry who underwent autologous or allogeneic transplantation for FL revealed that durable remissions could be induced with either technique. A lower 5-year recurrence rate with allogeneic transplantation was offset by a higher treatment-related mortality (TRM) compared with autologous transplantation, leading to similar 5-year survival rates of 51% to 62%. To reduce the TRM of allo SCT, a nonmyeloablative strategy is preferred in FL. Results utilizing a nonmyeloablative allogeneic SCT strategy vary widely in the literature. For example, a series of 62 patients treated at the Fred Hutchinson Cancer Research Center demonstrated a 3-year OS and PFS of 67% and 54%, respectively. Alternatively, a highly selected group ($n=47$) treated at the MD Anderson Cancer Center achieved an 11-year OS and PFS of 78% and 72%, respectively.

There is one small, randomized clinical trial (the CUP trial) examining ASCT versus standard therapy in patients with relapsed FL. The study, conducted in the pre-rituximab era, found improved PFS and a trend toward improved OS. An interesting long-term analysis of patients receiving myeloablative chemotherapy followed by ASCT comes from investigators at St. Bartholomew's Hospital (London) and the Dana-Farber Cancer Institute (Boston). A cohort of 121 patients, with a median follow-up of 13.5 years, was noted to have a plateau in the remission-duration curve beginning around year 8. Nearly half the patients were still in remission at 10 to 15 years, suggesting some patients may be cured. Results were substantially better for patients treated in second remission as opposed to later in the disease course, suggesting there may be an optimal window to consider ASCT in FL.

Patients who relapse within 2 years of their initial chemoimmunotherapy are at high risk of dying from FL with

a 5-year OS of approximately 50%. Retrospective analyses have been conducted to see if these high-risk patients might benefit preferentially from ASCT in the management of their relapsed disease. Data from the National Lympho-Care Study and Center for International Bone Marrow Transplant Research (CIBMTR) indeed showed no benefit in OS among all FL patients undergoing ASCT but did show an improved OS in the subgroup of patients with early progression of disease.

Marginal-zone lymphomas

The WHO classification separates the marginal-zone B-cell lymphomas (MZL) into extranodal MZL of MALT type, nodal MZL, and splenic MZL (SMZL). The morphology of these disorders is characterized by an infiltrate of centrocyte-like small cleaved cells, monocytoid B cells, or small lymphocytes; these disorders may exhibit an expanded marginal zone surrounding lymphoid follicles. The immunophenotype is characterized by expression of CD20 but lack of CD5 or CD10 expression (Table 23-2); this marker profile is useful in distinguishing MZL from SLL, MCL, and FL. A feature common to many cases of MZL is association with chronic antigenic stimulation by microbial pathogens or autoantigens as described above. Examples include gastric MALT (*H pylori*), cutaneous MALT (*B burgdorferi* or *afzelii*), ocular adnexal MALT (*C psittaci*), nodal MZL (hepatitis C), SMZL (hepatitis C), pulmonary or parotid MALT (Sjögren syndrome), and thyroid MALT (Hashimoto thyroiditis). There is significant geographic variation associated with certain microbial pathogens. For example, the prevalence of *C psittaci* in patients with ocular adnexal MALT appears to be 50% to 80% in Italy, Austria, Germany, and Korea, whereas this organism is observed infrequently in Japan, China, and the United States.

MALT lymphomas

Extranodal MZLs or MALT lymphomas constitute ~70% of all MZLs. They occur most commonly in mucosal sites, predominantly gastric or intestinal, as well as lung, salivary gland, ocular adnexa, skin, and thyroid, among others. These sites often are affected by chronic infection or inflammation in the setting of autoimmune disease, such as Sjögren syndrome or Hashimoto thyroiditis. The typical presentation of MALT lymphoma is an isolated mass in any of these extranodal sites or an ulcerative lesion in the stomach. Clinically, these lymphomas are typically indolent, with 10-year OS rates in excess of 90% in many series. MALT lymphomas can be characterized as gastric (30%-40%) or nongastric (60%-70%), and the approach to

disease management is site-specific. Approximately 90% of gastric MALT lymphomas are associated with *H pylori* infection. Newly diagnosed patients typically report dyspepsia, pain, reflux symptoms, or weight loss. Upper endoscopy can reveal erythema, erosions, ulcers, or masses. A consistent observation has been that 70% to 80% of gastric MALT lymphomas durably regress following effective *H pylori* antibiotic therapy. The most widely used antibiotic regimen is a combination of amoxicillin, omeprazole, and clarithromycin. Metronidazole is an effective alternative antibiotic in patients with a penicillin allergy. Lymphoma responses can be slow, taking as long as 6 months to 1 year. Repeat assessment of *H pylori*, by histologic examination or a urea breath test, is necessary to ensure that the bacteria have been eradicated. The strongest predictor for lymphoma nonresponse to antibiotic therapy is the presence of the t(11;18) translocation, which is present in 20% to 30% of cases. In the series reported by Nakamura et al, only 3 of 30 patients with t(11;18) experienced lymphoma regression following *H pylori* eradication therapy. In patients who do not respond to antibiotics, or in *H pylori*-negative cases, involved-field radiotherapy (IFRT) has been highly effective with DFS or PFS rates of >90% at 10 years. The prognosis for early-stage gastric MALT is excellent, with most series reporting 10-year OS rates in excess of 90%. For patients with advanced-stage disease, regimens similar to those used in FL, including rituximab alone or in combination, can be used. Transformation to DLBCL is possible, but a remarkable observation has been the regression of early-stage *H pylori*-positive gastric-diffuse large B-cell lymphomas with *H pylori*-eradication therapy. This observation was noted in DLBCL clearly arising from gastric MALT (transformation) and in de-novo DLBCL (no apparent underlying MALT).

Nongastric MALT lymphomas also have an indolent course, including the one-third of patients who present with stage 4 disease. OS at 10 years exceeds 90% in many series. The most common locations are the salivary glands (26%), ocular adnexa (17%), skin (12%), lung (8%), upper airways (7%), thyroid (6%), and intestinal tract (5%). Treatment approaches depend on both stage and site of primary involvement and may include surgery, radiation therapy, or chemotherapy. Radiation therapy produces excellent results in limited-stage disease. Many patients can be managed with surveillance alone if asymptomatic. Patients with advanced-stage disease typically can be managed using the same principles used for FL. Patients often have a low disease burden, and rituximab monotherapy may be highly effective. For high-tumor-burden patients or those progressing on rituximab alone, rituximab added

to chlorambucil was shown to improve EFS in an RCT compared to chlorambucil alone. Recurrences tend to occur in the same or other extranodal locations. For patients requiring chemoimmunotherapy, bendamustine has been employed with success, as with FL. Recently the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib was FDA-approved for relapsed/refractory marginal-zone lymphoma based on a 63-patient phase 2 trial for relapsed/refractory marginal-zone lymphoma of any subtype. The oral dose was 560 mg daily. Ibrutinib produced an ORR of 48% with a median PFS of 14.2 months, making this an appealing available option for patients with relapsed marginal zone lymphoma.

Nodal MZL

Nodal MZL also arises from marginal-zone B cells but presents with nodal involvement akin to FL. Whenever nodal MZL is diagnosed, a careful history review and a physical examination should be conducted to determine if a coexisting extranodal MALT lymphoma component exists, as concurrent disease may be present in up to one-third of cases. Nodal MZL more commonly presents at advanced-stage (Ann Arbor stage III-IV) than with MALT-type MZL. The t(11;18) karyotypic changes identified in MALT are absent in nodal MZL, and no specific or recurring karyotypic anomaly has been described. IgM monoclonal gammopathy can occur in ~10% of cases. HCV infection is reported in up to 25% of patients. Across reported series, the 5-year OS for nodal MZL is 60% to 70%; however, the EFS is only 30%, which likely reflects more commonly encountered advanced-stage disease. Management is similar to the approach recommended in FL, and ibrutinib is available as an option at relapse, as reviewed above.

In the updated WHO classification, a new category, pediatric nodal MZL, which has distinctive clinical and morphologic characteristics, was introduced. There is a male predominance (20:1), and patients usually present with localized asymptomatic adenopathy in the head and neck region. Morphologically, the infiltrate is similar to that seen in adults, except that progressively transformed germinal centers often are seen.

Splenic MZL

Splenic MZL (SMZL) presents at a median age of 68 years and is more common in females. Patients usually present with symptomatic splenomegaly, and involvement of the peripheral blood and bone marrow are common. Generalized lymphadenopathy is rare, but patients may have splenic hilar nodal or hepatic involvement. Patients may

have concomitant autoimmune cytopenias, which should be considered in patients with anemia or thrombocytopenia at diagnosis. Diagnosis usually is based on spleen histology following splenectomy or after bone-marrow examination. Clinically, SMZL can be confused with CLL, MCL, FL, HCL, or WM. Unlike CLL and MCL, SMZL is typically CD5-negative, and, unlike FL, it is CD10-negative. Unlike HCL, which is CD103-positive and replaces the splenic red pulp, SMZL is CD103-negative and replaces the splenic white pulp. WM may be distinguished from SMZL based on the presence of a *MYD88* mutation which does not occur in MZL. A prognostic model, using hemoglobin <12 g/dL, elevated LDH, and albumin <3.5 g/dL, has identified three distinctive risk groups (low, intermediate and high). OS at 5 years was 88%, 73%, and 50% for patients with 0, 1, and 2 or 3 risk factors, respectively, in the pre-rituximab era. All patients should be checked for underlying hepatitis C because antiviral therapy for hepatitis C often leads to regression of the SMZL and is the recommended initial treatment of choice in these patients. For non-hepatitis C patients, observation alone is the recommended initial approach for asymptomatic patients without bulky splenomegaly or significant cytopenias. For patients requiring therapy, splenectomy has long been considered the optimal first-line treatment. However, single-agent rituximab is also remarkably active, with an ORR approaching 100% in small series. In an observational retrospective study, rituximab produced more durable remissions than did splenectomy. For young patients, who are appropriate surgical candidates, splenectomy or rituximab monotherapy may be considered as initial therapy, whereas for elderly patients or patients otherwise unfit for surgery, rituximab monotherapy is preferred. Patients with subsequent relapses in need of therapy may be considered for splenectomy if not yet performed, retreatment with single-agent rituximab, or treatment with chemoimmunotherapy or ibrutinib.

Lymphoplasmacytic lymphoma and Waldenström macroglobulinemia

Lymphoplasmacytic lymphoma (LPL) is defined in the WHO classification as an indolent neoplasm of small B lymphocytes, plasmacytoid lymphocytes, and plasma cells. The lymphoma cells may express B-cell markers CD19 and CD20 and are CD5- and CD10-negative, much like the MZLs (Table 23-3). LPL with production of an IgM paraprotein produces the syndrome known as Waldenström macroglobulinemia, which is described further in Chapter 25.

Hairy cell leukemia

HCL is an indolent B-cell lymphoproliferative disorder accounting for only 2% of all leukemias; it is characterized pathologically by neoplastic lymphocytes with cytoplasmic “hairy” projections on the cell surface, a positive tartrate-resistant acid phosphatase stain, and an immunophenotype positive for surface immunoglobulin, CD19, CD20, CD22, CD11c, CD25, and CD103 (Table 23-2). Marrow biopsy demonstrates a mononuclear cell infiltrate with a “fried egg” appearance of a halo around the nuclei and increased reticulin and collagen fibrosis. Nearly 100% of cases harbor the *BRAF* V600E mutation, abnormally activating the *BRAF*-MEK-ERK pathway.

HCL is 4 times more common in men than in women and presents at a median age in the 50s with pancytopenia and splenomegaly. Most patients have an absolute monocytopenia, which may be a clue to the diagnosis. The bone marrow aspirate is often a dry tap due to increased marrow reticulin. Making the proper diagnosis is crucial because of HCL’s generally favorable prognosis, with a 10-year OS exceeding 90% and an excellent treatment response to nucleoside analogs. Most patients with HCL require therapy to correct cytopenias and associated complications, in addition to the presence of symptomatic splenomegaly. If a patient is asymptomatic and cytopenias are minimal, the patient may be observed initially. HCL is uniquely sensitivity to purine analogs. The nucleoside analogs cladribine or pentostatin are the treatments of choice in HCL in view of the high response rates and durable remissions achieved. Cladribine is used more commonly because of the short duration of therapy required; cladribine also is available as a subcutaneous injection. In one large series of 233 patients with long-term follow-up, the ORR and CR rates with either of these agents were 97% and 80%, respectively. The median recurrence-free survival was 16 years, and many of the relapses were observed 5 to 15 years after initial treatment, highlighting the unique natural history of this disease. It currently is recommended that assessment of response should be determined 4 to 6 months after the end of treatment; a second course can be given only if a PR is attained. Patients who relapse after frontline nucleoside analogue therapy are often retreated with a nucleoside analogue with similarly high response rates. Rituximab may also be administered for relapsed disease. For multiply relapsed patients, the anti-CD22 antibody drug conjugate moxetumomab pseudotox-tdfk is FDA approved for HCL relapsed after at least 2 prior therapies including a purine analog. Among 80 patients treated, the ORR was 75%, and the rate of durable CR (at least 180 days) was 30%. For the uncommon patients with relapsed HCL, who

are refractory to both nucleoside analogues and rituximab, *BRAF* inhibitors have also demonstrated high response rates as single agents and should be considered in these selected cases.

HCL-variant is a distinct disease categorized separately in the WHO classification, and, despite its name, it is considered to be unrelated to HCL. HCL-variant does not harbor the *BRAF*-V600E mutation. It differs from HCL in the lack of monocytopenia and by the presence of an elevated white blood cell count. The bone marrow is easier to aspirate because the reticulin fiber content is low. The immunophenotype of HCL-variant also differs in that the cells are CD25-negative. CD103 is expressed infrequently and CD11c is usually positive. Unlike HCL, HCL-variant responds poorly to purine analogs. Splenectomy can result in partial remissions, and some patients can respond well to rituximab.

Transformation to aggressive lymphoma in indolent lymphomas

Histologic transformation (HT) is the development of aggressive NHL in patients with an underlying indolent lymphoma. It most commonly occurs in FL but can occur in any of the indolent lymphomas. The British Columbia Cancer Agency reported on the incidence and outcome of 600 patients with FL who subsequently developed transformed lymphoma. Diagnoses were made clinically (sudden increase in LDH >2× the upper limit of normal, discordant nodal growth, or unusual extranodal sites of involvement) (37%) or pathologically (63%). In this series, the annual risk of transformation was 3% per year, with 10- and 15-year risks of 30% and 45%, respectively. Overall, the median post-transformation survival time was 1.7 years, with superior outcomes observed in limited-stage patients. Similar results were observed in a series from St. Bartholomew, where histologic transformation was observed in 28% of patients with FL by 10 years. A more recent analysis in the rituximab era, however, demonstrates a lower overall rate of HT in FL of 15% and with an improved outcome. FDG-PET imaging can be helpful in selecting a biopsy site when establishing HT, but bright FDG avidity alone does not establish a diagnosis of HT. Histologically, DLBCL is the most frequently observed subtype. One should assay for MYC and BCL-2 by FISH and by immunohistochemistry. The treatment is directed at the aggressive lymphoma and depends on a variety of factors, including age, comorbidities, and extent of prior treatment for FL. Patients with HT, who have never received R-CHOP, have a cure rate similar to de novo DLBCL, making R-CHOP the treatment of choice in most patients. Consideration for stem-cell transplantation consolidation is warranted in selected patients.

KEY POINTS

- Follicular NHL is the most common indolent NHL.
- Patients with asymptomatic, advanced-stage indolent NHL may be followed without specific therapy to assess the pace of disease, or single-agent rituximab may be used to delay the use of systemic chemotherapy.
- Anti-CD20 antibody therapy plus chemotherapy is recommended in patients with symptomatic or high-tumor-burden disease by the GELF criteria.
- Maintenance anti-CD20 antibody therapy improves PFS with no impact on OS.
- There are a multitude of therapeutic options for relapsed indolent lymphoma, including novel targeted agents and stem-cell transplantation.

DLBCL constitutes approximately 30% of all NHLs and can present with nodal or extranodal disease. Bone-marrow involvement with large-cell lymphoma occurs in fewer than 10% of cases. Another 10% to 20% of patients have discordant marrow involvement with a low-grade B-cell lymphoma, despite a nodal biopsy consistent with DLBCL.

In addition to the B-cell markers CD20 and CD19, the neoplastic cells may also express CD10 (30% to 60%), BCL6 (60% to 90%), and IRF4/MUM1 (35% to 65%). Rare cases may express CD5 (10%) and must be distinguished from the blastoid variant of MCL, which is cyclin-D1-positive. As described, two molecularly distinct subtypes of DLBCL NOS are recognized: GCB, which has a gene-expression profile similar to germinal-center B cells (CD10⁺ and BCL6⁺); and activated B-cell (ABC), which has a profile similar to activated peripheral B cells (IRF4/MUM⁺) with a prominent *NFKB* gene signature.

Aggressive B-cell lymphomas

DLBCL is the prototypical aggressive lymphoma, with other histologies including MCL, BL, peripheral T-cell lymphomas, anaplastic large-cell lymphoma, and others (Table 23-3). These neoplasms are typically characterized by rapidly progressing nodal or extranodal disease and, although often potentially curable, are associated with relatively short survival in the absence of successful therapy. This chapter focuses on the mature B- and T-/NK-cell neoplasms.

CLINICAL CASE

A 52-year-old man is diagnosed with stage IVB DLBCL. On PET-CT imaging, the largest nodal mass was 6 cm in the retroperitoneal region, and there was lymphoma involvement of liver and bone. Laboratory studies show a normal complete blood count (CBC) and normal chemistries, aside from an LDH elevated 1.5 times normal. His Eastern Cooperative Oncology Group performance status (ECOG PS) is 1. Immunophenotypic stains of the lymphoma cells revealed expression of CD19, CD20, κ light chains, BCL2, MYC, and MUM1/IRF4. Lymphoma cells were negative for CD10 and BCL6 expression.

Clinical prognostic factors in DLBCL

Approximately two-thirds of patients diagnosed with DLBCL can be cured with rituximab-based chemotherapy; however, low- and high-risk groups can further be defined by clinical and biological factors. Although the IPI is robust and relevant in the modern rituximab treatment era, it does not capture all prognostic information. The patient described earlier has an IPI score of 3 (advanced-stage, multiple sites of extranodal involvement, elevated LDH), placing him in a high-intermediate-risk group with an expected 5-year probability of survival with R-CHOP of 50% to 60%.

Biological prognostic factors in DLBCL

Although the IPI is easy to apply and remains valid in the current treatment era, it fails to capture underlying biological heterogeneity. As described above, DLBCL can be divided molecularly by gene-expression profiling (GEP) into the germinal-center B-cell (GCB) and activated B-cell (ABC) subtypes, which also have a signature distinct from PMBCL. ABC DLBCL has an inferior prognosis, independent of the IPI. The use of GEP has had limited clinical utility due to long turnaround time, the need to use fresh frozen tissue, technical complexity, and lack of routine availability in the clinic.

Immunohistochemical (IHC) algorithms have been used in an attempt to capture the cell-of-origin (COO) phenotype using a methodology that can be applied routinely in clinical practice. Hans et al first reported an IHC algorithm to distinguish the GCB versus non-GCB subgroups using CD10, BCL6, and IRF4/MUM1. Using the cDNA microarray as the gold standard, the sensitivity of the IHC COO subgrouping was 71% for the GCB group and 88% for the non-GCB group. Other algorithms have

Diffuse large B-cell lymphoma

DLBCL is composed of large B cells with a diffuse growth pattern. The WHO classification recognizes several sub-categories of DLBCL, including molecular subtypes (GCB and ABC; see later sections); pathologic subtypes, including T-cell/histiocyte-rich large B-cell lymphoma; and defined disease entities, including primary mediastinal large B-cell lymphoma (PMBCL) and primary DLBCL of the CNS.

been proposed that also have a lower sensitivity than gene-expression profiling. These results, however, have been inconsistent as to whether the COO distinction by IHC can be applied to rituximab-treated patients. One study found that none of the applied five different IHC algorithms could distinguish COO subgroups with prognostic significance. In contrast, another study found that the Tally algorithm, which uses CD10, GCET, IRF4/MUM1, and FOXP1, showed the best concordance with microarray data and maintained prognostic significance. Given these inconsistencies and the lack of data suggesting that alternate therapies may affect outcome, the COO information, whether by molecular profiling or immunohistochemistry, should not be used to direct treatment decisions outside of clinical trials.

Recent technological advances in GEP, allows real-time COO determination from formalin-fixed paraffin-embedded tissue (FFPET). The Lymphoma/Leukemia Molecular Profiling Project developed the Lymph2Cx assay, a parsimonious digital gene-expression (NanoString)-based test for COO assignment in FFPET. A 20-gene assay was trained using 51 FFPET biopsies, and the locked assay was subsequently validated using an independent cohort of 68 FFPET biopsies. Comparisons were made with COO assignment using the original COO model on matched frozen tissue. The assay was highly accurate; only 1 case with definitive COO was incorrectly assigned with >95% concordance of COO assignment between two independent laboratories. The test turnaround time is several days, making Lymph2Cx attractive for implementation in clinical trials and practice. However, until gene-expression analysis becomes clinically available, the 2016 WHO classification includes subclassification of DLBCL NOS as GCB or non-GCB based on IHC algorithms.

MYC is translocated in ~5% to 10% of DLBCLs, and early studies have suggested that *MYC* is associated with an aggressive course in the pre- and post-rituximab treatment eras. In some cases, there is also a t(14;18) involving *BCL2*, or a *BCL6* translocation involving chromosome 3, in which case the disease has been dubbed double-hit lymphoma (DHL) or triple-hit lymphoma (THL), if all three translocations are present. DHL/THL can occur as a high-grade transformation from an underlying FL or as a de-novo disease. The combination of *MYC* driving cellular proliferation and *BCL2* preventing apoptosis has proven to be an extremely high-risk biologic subset of aggressive lymphomas with low cure rates using traditional R-CHOP. In the previous 2008 WHO classification scheme, DHLs were incorporated within the classification of DLBCL or, more commonly, of B-cell lymphoma, unclassifiable with features intermediate between BL and diffuse large B-cell

lymphoma. In the 2016 WHO, DHL is now a distinct molecularly defined aggressive lymphoma called “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements” and the previous classification of “B-cell lymphoma, unclassifiable with features intermediate between BL and diffuse large B-cell lymphoma” has been eliminated. With the recent availability of a *MYC* antibody for IHC analysis, two large-scale studies have evaluated the prognostic importance of *MYC*- and *BCL2*-protein expression (double expressers) in DLBCL patients treated with R-CHOP chemotherapy. *MYC* protein expression was found in approximately one-third of cases, a higher incidence than that captured by fluorescence in situ hybridization (FISH) analysis (11%) or high *MYC* mRNA expression, suggesting that multiple roads of *MYC*-deregulation exist. Importantly, the double expressers, which account for 20% to 25% of newly diagnosed DLBCLs, have an inferior prognosis relative to other DLBCLs, though not as poor as for patients with DHL. Novel treatment approaches for these high-risk patients are needed.

Treatment of newly diagnosed DLBCL

Advanced-stage DLBCL

The backbone of treatment of all subtypes of DLBCL is anthracycline-based treatment with R-CHOP chemotherapy. With this approach, approximately two-thirds of patients are cured.

Rituximab has several mechanisms of action, including the ability to sensitize otherwise-resistant lymphoma cells to chemotherapy agents in vitro, perhaps, in part, via downregulation of the BCL-2 protein. GELA published a landmark phase 3 clinical trial in which 399 patients 60 to 80 years of age, with previously untreated advanced-stage CD20+-DLBCL, were randomized to receive CHOP for 8 cycles or R-CHOP on a standard 21-day schedule. R-CHOP demonstrated an improvement over CHOP for all endpoints, including CR rate, EFS, and OS. With longer follow-up, the results held, and R-CHOP quickly became the standard of care for advanced-stage DLBCL around the world (Table 23-12). More recently, the median 10-year-outcome of patients in this study demonstrated a 10-year PFS for R-CHOP-treated patients of 35% (vs 20% for CHOP alone) and a 10-year OS of 43.5% (vs 27.6% for CHOP alone) (Table 23-12). A similar phase 3 study was carried out by the US ECOG intergroup (E4494) study comparing 6 to 8 cycles of CHOP versus R-CHOP in elderly patients with aggressive lymphoma, which included a second randomization in CR patients comparing observation and rituximab maintenance therapy every 6 months for 2 years. Unlike the GELA study, there was no response-rate or OS difference detected, although

Table 23-12 Key trials of diffuse large B-cell lymphoma using rituximab-containing regimens

Author (trial/phase)	N	Treatment	Patient selection	PFS/EFS	OS
Coiffier et al, <i>N Engl J Med.</i> 2002 (GELA/III)	202	R-CHOP×8 vs	Age 60–80 y Stage II-IV	57% vs 38% (2 y)	70% vs 57% (2 y)
	197	CHOP×8			
Pfreundschuh et al, <i>Lancet Oncol.</i> 2006 (MInT/III)	413	R-CHOP-like [‡] ×6 vs	Age 18–60 y aaIPI 0 or 1 Stage I (+bulk or II-IV)	74% vs 56% (6 y)	90% vs 80% (6 y)
	410	CHOP like [‡] ×6			
Pfreundschuh et al, <i>Lancet Oncol.</i> 2008 (RiCOVER-60/III) [†]	306	R-CHOP-14×6	Age 61–80 y Stage I-IV	66.5% (3 y) 63% (3 y)	78% (3 y) 72.5% (3 y)
	304	R-CHOP-14×8			
	209	CHOP-14×6		47% (3 y)	68% (3 y)
	219	CHOP-14×8		53% (3 y)	66% (3 y)
Cunningham et al, <i>Lancet</i> 2013 (NCRI/III)	540	R-CHOP-21×8	Age 61–80 y	81% vs 83% (2 y)*	81% vs 83% (2 y)*
	540	R-CHOP-14×6+ G-CSF			
Delarue et al, <i>Lancet Oncol.</i> 2013 (LNH03-6B/III)	296	R-CHOP-21×8	Age 60–80 y aaIPI >1	60% vs 56% (3 y)*	72% vs 69% (3 y)*
	304	R-CHOP-14×6			
Recher et al, <i>Lancet</i> 2011 (LNH03-2B/III)	196	R-ACVBP	Age 18–59 y aaIPI 1	87% vs 73% (3 y)	92% vs 89% (3 y)
	183	R-CHOP			

Survival estimates shown for rituximab-containing regimens only and are rounded off where applicable to the nearest whole number.

EFS, event-free survival; G-CSF, granulocyte colony-stimulating factor; GELA, Groupe d'Etude des Lymphomes de l'Adulte; MInT, MabThera International Study Group; NCRI, British National Cancer Research Institute Study; R, rituximab; RiCOVER-60, Rituximab with CHOP Over Age 60 Years.

*87% DLBCL; CHOP-like = CHOP-21 or CHOEP-21 in 92%; radiotherapy given to sites of bulk, extranodal disease (physician's discretion).

[†]80% DLBCL.

*P value not significant (all other P values for comparisons are significant).

there was a benefit in TTF for the R-CHOP arm. The analysis was confounded to some extent by the secondary randomization to maintenance vs no-maintenance rituximab. Maintenance therapy was beneficial for the TTF only in the CHOP-induction subset. As such, interpretation of these results supports the use of R-CHOP induction without subsequent maintenance rituximab therapy.

Two other randomized controlled studies have been published supporting the benefit of the addition of rituximab to anthracycline-based chemotherapy in DLBCL. The MabThera International Study Group (MInT) study included young (<60 years), low-risk (aaIPI 0 or 1) patients with DLBCL (including PMBCL) who primarily received CHOP or CHOP plus etoposide (CHOEP) with or without rituximab. The rituximab-containing regimens demonstrated an improvement in EFS and OS (Table 23-12). The RItuximab with CHOP OVER age 60 Years (RICOVER-60) trial by the same group evaluated CHOP-14 for 6 or 8 cycles, with or without rituximab in elderly patients and also demonstrated a significant improvement in all endpoints with the rituximab combinations. Of note, the latter study also established that 6 cycles of R-CHOP-14 was associated with the best outcome.

Two randomized studies (GELA LNH-03-6B and the British National Cancer Research Institute [NCRI]) compared R-CHOP-21 (ie, every 21 days) with R-CHOP-14 (every 14 days), and there was no improvement of FFS or OS using the shortened cycle interval, thus confirming that R-CHOP-21 remains the standard (Table 23-12). Based upon the observation that elderly females fare better with R-CHOP than do elderly males and that elderly males clear rituximab more rapidly, dose-dense rituximab regimens are being tested in elderly males. A trial, where elderly males were treated with higher dose of rituximab given at 500 mg/m² while females received standard dose of 375 mg, showed that outcomes for male patients treated with higher-dose rituximab was equivalent to outcomes of historically treated females. Several recent randomized trials have sought to improve upon R-CHOP results in DLBCL. Explored strategies compared to standard R-CHOP have included substituting the next generation anti-CD20 monoclonal antibody obinutuzumab for rituximab, addition of the proteasome inhibitor bortezomib, maintenance everolimus, consolidation with HDC and ASCT, and infusional therapy with dose-adjusted EP-OCH-R. All randomized trials showed no improvement

in survival over standard R-CHOP. Based on these data, administration of R-CHOP every 21 days for 6 cycles remains the standard of care for advanced-stage DLBCL.

Treatment of limited-stage DLBCL

Approximately 45% of cases of DLBCL are limited-stage, Ann Arbor stages I-II. A large randomized Southwest Oncology Group (SWOG) trial (SWOG-8736) in the pre-rituximab era established that CMT, including chemotherapy followed by radiation, was superior to CHOP alone for localized [stage I(E), nonbulky stage II(E)] aggressive lymphoma. In this study, the 5-year PFS (77% vs 65%, $P=0.03$) and OS (82% vs 72%, $P=0.02$) for three cycles of CHOP followed by IFRT was superior to that of 8 cycles of CHOP alone. An update of the study with longer follow-up, however, showed that the treatment advantage for the CMT was not sustained; there was an identical 10-year PFS of 55% in both treatment arms.

The benefit of rituximab has not been specifically analyzed in a randomized controlled trial in localized DLBCL. The majority of patients in the MInT study had limited-stage disease by nature of the inclusion criteria, and that study confirmed the benefit of rituximab in this population. The SWOG completed a phase 2 study evaluating 3 cycles of R-CHOP, with 4 doses of rituximab, followed by IFRT (40–46 Gy, if CR, and 50–55 Gy, if PR) in patients with localized aggressive B-cell lymphoma, most of whom had DLBCL. Patients had to have at least one risk factor by the stage-modified IPI and had a 10-year PFS and OS of 58% and 67%, respectively.

With potential acute and more concerning long-term side effects of radiotherapy, determining whether a subgroup of patients with limited-stage DLBCL can be selected to receive chemotherapy alone is an important issue. A French study in limited-stage nonbulky (<7 cm) DLBCL randomized patients to 4–6 cycles of R-CHOP followed by 40 Gy XRT or to 4–6 cycles of R-CHOP alone. Patients with an IPI score of 0 received 4 cycles, while patients with IPI scores of ≥ 1 received 6 cycles. Only patients in a CR by PET-CT were randomized between chemotherapy alone or CMT, while all PR patients received CMT. Eighty-eight percent of patients achieved a CR and were randomized, with no difference in 5-year EFS or OS between the treatment arms. These data validate chemoimmunotherapy alone as an appropriate treatment plan for nonbulky limited-stage DLBCL patients who achieve a CR to R-CHOP.

CR patients received 4 to 6 cycles of R-CHOP followed by 40-Gy RT. Patients in CR by PET imaging after 4 cycles (84%) did not receive cycles 5 and 6 of R-CHOP.

The patients assigned to no RT had EFS and OS that were not different compared to patients receiving RT, suggesting RT may be unnecessary in selected patients responding well to chemoimmunotherapy alone.

Primary testicular DLBCL represents a unique subset of DLBCL, most commonly presenting at limited-stage. These patients have a propensity for late relapse, as well as a high risk of CNS recurrence (parenchymal > leptomeningeal) and recurrence within the contralateral testis. As such, patients with primary testicular DLBCL are typically treated with 6 cycles of R-CHOP, including CNS prophylaxis, followed by prophylactic scrotal radiation to the contralateral testis.

Novel strategies to improve cure rates in DLBCL

Although the outcome of DLBCL has improved with R-CHOP chemotherapy, ~43% of patients still relapse after primary therapy, and most relapsing patients will not be cured of their disease. As noted earlier, multiple randomized trials have failed to identify therapy superior to R-CHOP. Ongoing trials are now seeking to incorporate novel target agents with a biologic rationale in discrete DLBCL subsets. Both lenalidomide and ibrutinib may be selectively beneficial in ABC-DLBCL, with each showing single-agent activity in relapsed ABC-DLBCL compared to GCB. Randomized trials are currently evaluating each of these agents in combination with R-CHOP compared to R-CHOP alone, specifically in ABC/non-GCB DLBCL. Results of these trials are eagerly anticipated and could change the standard of care in a biologically defined subset of DLBCL patients.

Management of relapsed and refractory DLBCL

Repeating a biopsy at the time of suspected recurrence is recommended given the implications of recurrent DLBCL and possibility of relapse with a different histology. Following confirmation of recurrence, patients should undergo full restaging investigations. If the patient does not have significant comorbidities and is younger than 70 years of age (younger than 80 in some centers), second-line (salvage) combination chemotherapy, such as R-ICE (rituximab, ifosfamide, carboplatin, etoposide), R-DHAP (rituximab, dexamethasone, Ara-C, cisplatin), or R-GDP (rituximab, gemcitabine, dexamethasone, cisplatin) should be given followed by HDC/ASCT, if chemotherapy-sensitive disease is demonstrated. The evidence supporting the use of HDC/ASCT in relapsed DLBCL is based on the historic Parma study (named after the city of Parma, Italy where the study group who conducted the trial first met). Patients, who relapsed with aggressive lymphoma (excluding CNS or bone

marrow involvement) following an initial CR to primary therapy, received 2 cycles of DHAP chemotherapy. If chemosensitivity (ie, a PR or CR to salvage chemotherapy) was demonstrated, patients were then randomized to receive further chemotherapy with DHAP or with HDC with BEAC (carmustine, etoposide, cytarabine, and cyclophosphamide) and ASCT. Patients in the transplantation arm had an improvement in both the 5-year EFS (46% vs 12%, $P=.001$) and OS (53% vs 32%, $P=.038$). Randomized trials in the modern era, however, have demonstrated disappointing success rates with this approach in patients who relapse or are refractory to R-CHOP, with fewer than 30% of patients remaining progression-free at 2 years.

The optimal salvage therapy recently has been investigated in 3 phase 3 randomized controlled trials. The Collaborative Trial in Relapsed Aggressive Lymphoma (CORAL) study randomized patients with relapsed DLBCL (or those who had not achieved a CR) to receive rituximab plus ifosfamide, carboplatin, and etoposide (R-ICE) or rituximab plus dexamethasone, high dose ara-C, and cisplatin (R-DHAP) for 3 cycles followed by HDC with carmustine (BCNU), etoposide, cytarabine and melphalan [BEAM]/ASCT if a response was demonstrated. There was also a second randomization following transplantation to rituximab or to observation to evaluate the role of maintenance therapy. At diagnosis, 62% of the patients had been treated with a CHOP-like regimen with rituximab. The ORR was similar between R-DHAP and R-ICE (63% vs 63.5%), and there was no difference in EFS or OS, and maintenance rituximab did not affect outcome. Patients who previously had received rituximab with their primary therapy had an inferior response rate (51% vs 83%, $P<.001$) and an inferior 3-year EFS (21% vs 47%), suggesting that these patients represent a very chemoresistant group. Additional poor prognostic factors that emerged from this study were early relapse <1 year and an aaIPI of 2 or 3. Interestingly, a subsequent correlative study suggested that patients with GCB DLBCL had an improved outcome to R-DHAP compared with R-ICE (3-year PFS 52% vs 32%, $P=.018$), which was even more striking if cases were defined by gene-expression profiling (GEP) (3-year PFS 100 % vs 27%), but the numbers were small. A second phase 3 trial was conducted by the NCIC (National Cancer Institute of Canada) comparing R-DHAP to the outpatient salvage regimen R-GDP (rituximab, gemcitabine, dexamethasone, cisplatin) in aggressive lymphomas using a noninferiority design. The ORR, EFS, and OS were similar between the treatment arms, but the R-GDP arm was associated with less grade 3 or 4 toxicity ($P=.0003$), including febrile neutropenia

(9% vs 23%, $P<.0001$); patients had superior QOL scores. Finally, a third randomized trial evaluated ofatumumab-DHAP vs R-DHAP as salvage therapy prior to ASCT in relapsed DLBCL and found no difference between the arms. The complete response rates to salvage therapy were low in both arms, and only 25% of patients remained progression-free at 2 years, highlighting treatment of relapsed DLBCL as a largely unmet medical need in the modern era. The primary predictor of success was achieving a CR by PET scan prior to ASCT.

Management of non-transplant-eligible patients with relapsed or refractory DLBCL, including novel therapies

Many patients relapse after HDC/ASCT or are not eligible for curative-intent treatment with salvage chemotherapy and HDC/ASCT due to advanced age or comorbidities. The goal of treatment in this setting is typically palliative; therefore lower intensity regimens are typically employed which may offer short-term disease control with modest treatment-associated toxicity. Commonly used regimens in this context include gemcitabine-based regimens, such as R-GemOx (rituximab, gemcitabine, oxaliplatin), or rituximab-bendamustine. Certain therapies may also be appealing in selected subsets of relapsed/refractory DLBCL. For tumors expressing CD30, the anti-CD30 antibody drug-conjugate brentuximab vedotin produces an overall response rate of 44% with a median duration of response of approximately 6 months and should be considered as an option in relapsed/refractory CD30⁺ DLBCL. Lenalidomide monotherapy produces responses in approximately one-quarter of relapsed DLBCL patients, but the response rate and durability represent the subset of patients with non-GCB DLBCL for whom this therapy should be considered. Similarly, the BTK inhibitor ibrutinib produces selectively higher responses in the ABC subset of DLBCL in whom the ORR was 37%. Interestingly, the pattern of mutations within the ABC DLBCL may help predict patients likelier to respond to ibrutinib. Patients harboring mutations of both *CD79B* and *MYD88* appear to have the highest likelihood of response, while *CARD11* and *TNFAIP3* mutations appear unlikely to respond.

Most recently, genetically modified autologous chimeric-antigen-receptor (CAR) T cells targeting CD19 have emerged as highly active agents in the management of chemotherapy-refractory DLBCL. The anti-CD19 CAR T-cell product axicabtagene ciloleucel (axi-cel) was evaluated in a phase 2 trial of 111 patients with chemotherapy-refractory DLBC, PMBCL, or transformed FL. Refractoriness to chemotherapy was defined as lack of response

to prior therapy or relapse within 1 year of HDC/ASCT. The median number of prior therapies was 3, and 21% had relapsed after ASCT. Among 111 enrolled patients, 101 patients were treated with axi-cel, while the remaining 10 subjects did not receive their infusion due to adverse events (4), lack of measurable disease (2), death from disease progression (1), and manufacturing failure (1). The overall response rate for treated patients was a remarkable 82%, with a complete response rate of 54%. At 1 year of follow-up, 42% of subjects remained in remission, demonstrating encouraging durability in a significant proportion of these high-risk patients. Toxicities from CAR T cells include cytopenias resulting from the lymphodepleting fludarabine and cyclophosphamide which precedes the CAR T-cell infusion, as well as toxicities related to cytokine release in the setting of in-vivo CAR T-cell expansion. Cytokine release syndrome (CRS) was observed in 93% of patients treated with axi-cel and was most commonly characterized by fever, hypoxia, and hypotension. CRS was severe (grades 3–4) in 13% of patients, and was almost entirely reversible, although there were 2 deaths. The syndrome is largely driven by release of IL-6, and treatment with the IL-6 receptor antagonist tocilizumab does help to rapidly reverse the syndrome in most patients without impairing efficacy of the treatment. The other common toxicity was a neurologic event, which occurred in 64% of patients (28% severe) and was most commonly encephalopathy, aphasia, or somnolence. As with CRS, most cases are entirely reversible, with steroids appearing to be the most effective therapy in severe cases. Based on these data, axi-cel was FDA-approved for DLBCL, PMBCL and transformed FL patients who had received at least 2 prior lines of therapy and is now the most effective therapy available for chemotherapy-refractory DLBCL. Given the complexity and toxicity profile of this therapy, it must be administered only at centers experienced in its use. Tisagenlecleucel is another recently FDA-approved anti-CD19 CAR T-cell for multiply relapsed or refractory DLBCL and transformed FL, with other products in development and likely to join the treatment armamentarium.

Special situations: management of specific clinicopathologic entities of DLBCL

Primary mediastinal (thymic) large B-cell lymphoma

PMBCL was recognized as a specific entity in the WHO classification based on unique clinicopathologic presentation. Unlike typical cases of DLBCL, PMBCL occurs at a median age of 35 years and is slightly more common in women than in men. Most patients present with a bulky anterior mediastinal mass that can invade the lung and

chest wall and occasionally can cause superior vena cava syndrome. Distant spread is uncommon at diagnosis, occurring in about one-quarter of patients. At relapse, involvement of visceral extranodal sites, including the kidneys, adrenals, ovaries, liver, and CNS, can occur.

Histologically, sclerosis is typically present, and phenotypically, the cells lack surface immunoglobulin expression but express B-cell markers, such as CD19 and CD20. CD30 expression is present in 80% of cases; however, it is usually weak and heterogeneous. Interestingly, gene-expression analysis has shown that PMBCL is molecularly distinct from typical DLBCL and shares many components of the molecular signature with cHL. It had long been speculated that there may be a pathogenic overlap between the nodular-sclerosis subtype of cHL based on shared clinical features, including a young age of onset and mediastinal predominance, as well as pathologic features, including predominant fibrosis and tumor cells that are CD30⁺. In addition, composite and sequential lymphomas have been reported, and a gray zone lymphoma (GZL) with overlapping features of both malignancies is now defined in the WHO classification (see the section “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL”), further highlighting the biological continuum between these diseases.

A novel recurrent translocation involving *CIITA* (MHC class II transactivator), found to be recurrent in PMBCL and occurring in 38% of patients, is also found in 15% of cHL (Table 23-2). Cases with these chromosomal breaks have an inferior disease-specific survival. Prior studies also found reduced expression of MHC class II genes, which also is linked to an inferior outcome. Additionally, PMBCL often has 9p24.1 amplifications that results in increased expression of PD-1 ligand, which is a rational therapeutic target (discussed below).

The outcome of patients with PMBCL is generally favorable, with a 5-year PFS of 70% when patients are treated with R-CHOP, though approximately 20% of patients have primary induction failure which can be very difficult to salvage. Given the typical bulky localized presentation, the majority of patients have historically also received consolidative radiation therapy, which exposes this population of predominantly young women to late radiation risks including breast cancer and heart and lung disease. The significant rate of primary refractory disease with R-CHOP and the need for radiation therapy in the majority of patients prompted evaluation of dose-adjusted etoposide + prednisone + vincristine + cyclophosphamide + doxorubicin + rituximab (DA-EPOCH-R) without radiation in a phase 2 study at the National Cancer Institute. Fifty-one patients, median age, 30 years, were treated. Fifty-nine

percent of patients were female, 65% had bulky disease ≥10cm, and 29% had stage IV disease. At a median follow-up of 5 years, 93% of patients were event-free, and the OS was 97%. These data have resulted in widespread adoption of DA-EPOCH-R without radiation therapy as the up-front treatment of choice for most patients with PMBCL.

Relapsed PMBCL is treated similarly to other relapsed DLBCLs, with second-line chemoimmunotherapy and HDC/ASCT being the treatment of choice for patients with chemosensitive disease. Unfortunately, PMBCL is often highly chemoresistant at the time of progression and has been historically very difficult to salvage with conventional therapy. For patients relapsing after ASCT, or not eligible for ASCT due to chemorefractory disease, the PD-1 inhibitor pembrolizumab has shown evidence of efficacy as has anti-CD19 CAR T-cell therapy, both of which are now available for chemotherapy-refractory PMBCL.

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL

Introduced in the WHO 2008 classification, this diagnosis was defined by overlapping clinical, morphological, or immunophenotypic features between cHL and DLBCL, particularly PMBCL. These cases of so-called GZL usually occur in young men between 20 and 40 years old who present with an anterior mediastinal mass and who may have supraclavicular lymph node involvement. A broad spectrum of cytological appearances can occur within the same tumor. The immunophenotype often is transitional between PMBCL and cHL (see Chapter 22) with the tumor cells CD45⁺, CD20⁺, CD30⁺, and CD15⁺. Cases of morphologically nodular sclerosis cHL with strong and uniform expression of CD20 and CD15 would favor a diagnosis of GZL. In contrast, cases resembling PMBCL but that are CD20⁻ and CD15⁺ or EBV⁺, also would support a diagnosis of GZL. Clinical outcomes appear inferior in GZL compared to PMBCL or HL, but higher remission rates have been observed with DLBCL-type regimens, such as R-CHOP or DA-EPOCH-R rather than Hodgkin lymphoma therapy. Given the increased risk of chemoresistance in this subset, consolidative radiation therapy should be considered in patients with localized disease.

T-cell/histiocyte-rich DLBCL

T-cell/histiocyte-rich DLBCL is an uncommon variant of DLBCL, which usually presents at advanced stage with frequent involvement of liver, spleen, and bone marrow. Typically, the neoplastic cells comprise <10% of cellular population and are outnumbered by a background of abundant T-cells and histiocytes. Histologically, it can resemble nodular lymphocyte predominant HL (NLPHL)

or can be transformed from a prior diagnosis of NLPHL. Treatment with R-CHOP leads to results similar to those seen in DLBCL NOS and remains the standard of care.

High-grade B-cell lymphoma with MYC and *BCL2* and/or *BCL6* rearrangements (double-hit lymphoma)

Five to 10% of DLBCL patients have DHL, defined as the presence of *MYC* and *BCL2* or *BCL6* translocations (detected by FISH or karyotype). These cases have mutational features, and frequently morphologic features, intermediate between DLBCL and BL and have been reclassified in the 2017 WHO classification as high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements. These high-risk patients have lower OS when treated with R-CHOP; therefore, R-CHOP is considered an inadequate therapy for the majority of patients with DHL, who have a median OS of approximately 2 years.

The majority of patients present with poor prognostic features, including advanced age, elevated LDH, and an advanced stage, often with extranodal involvement, including CNS. Patients may present with circulating leukemic-phase disease, which is extremely uncommon in typical cases of DLBCL. Due to inadequacy of R-CHOP therapy, various intensified chemoimmunotherapy strategies have been used, largely based on experience in BL; however, advanced age of most patients and often poor performance status limits the use of highly intensive chemotherapy. Due to rarity of DHL, data largely come from retrospective reviews, making comparison between regimens difficult. The intensified upfront induction regimens including R-HyperCVAD/MA and R-CODOXM/IVAC appear to compare favorably with historical controls treated with R-CHOP; however, one must bear in mind that patients who are candidates for such intensive therapy are frequently younger and have better PS; therefore, results may not be generalizable to a majority of patients with DHL. DA-EPOCH-R therapy does appear to perform better than R-CHOP in retrospective analyses and can be tolerated in older adults, leading to wide employment of this regimen for this disease. Given the high risk of CNS dissemination, prophylactic therapy for the CNS is recommended. Whether consolidative stem-cell transplantation offers additional benefit remains uncertain, but thus far retrospective analyses have not identified a clear benefit for transplantation in first remission for DHL. Novel agents for this disease are under investigation and are clearly needed. Encouragingly, patients with chemotherapy-refractory DHL have been shown to have responses to anti-CD19 CAR T-cell therapy analogous to patients with DLBCL NOS, and so should be considered for this treatment.

KEY POINTS

- Diffuse large B-cell lymphoma (DLBCL) is the most common histologic subtype of NHL.
- The IPI and cell-of-origin phenotype remain prognostic in the rituximab treatment era in DLBCL. Studies are ongoing to determine whether patients classified as high risk by the IPI or ABC phenotype should be treated with a therapy other than R-CHOP.
- Treatment with R-CHOP-21 (ie, repeated every 21 days) for 6 cycles is a standard of care in advanced disease; the role of consolidative radiation in advanced disease is not well defined.
- In limited-stage disease, abbreviated chemotherapy with 3–4 cycles of R-CHOP plus involved-field radiotherapy (IFRT) can be used. R-CHOP alone is an option for patients with nonbulky disease who achieve a CR on their PET-CT.
- Presence of relapsed disease should be documented by biopsy whenever possible.
- Transplantation-eligible patients with relapsed DLBCL are usually treated with salvage chemotherapy (RDHAP, RICE, and RGDP appear to have similar efficacy) followed by high-dose chemotherapy and stem-cell transplantation.
- Anti-CD19 CAR T-cell therapy can induce durable remissions in a significant proportion of chemotherapy-refractory DLBCL, PMBCL, and transformed FL.
- PMBCL patients should preferentially be treated with DA-EPOCH-R without RT, though there are no randomized studies in this disease.
- High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements (DHL) represents a particularly poor prognostic category when treated with R-CHOP; the disease is usually treated with more intensive regimens.

Primary CNS lymphoma

Primary CNS lymphoma (PCNSL) can occur in the brain parenchyma, spinal cord, eye (ocular) (Figure 23-3), cranial nerves, or meninges. Of note, although 95% of cases of PCNSL are DLBCLs, rare cases of peripheral T-cell lymphoma (PTCL), low-grade lymphoma, and BL also have been reported. In addition to B-cell markers, CD10 expression is observed in only 10% to 20% of cases, but BCL6 expression is common (60% to 80%). Most cases (>90%) are of the activated B cell-like (ABC) subtype of DLBCL. Mutations of *CD79B*, *MYD88*, and *PIM1* are frequently observed. Amplifications of 9p24.1 are common and result in PD-L1 expression in the majority of cases. PCNSLs are rare and may occur in immunocompetent patients or in association with immunosuppression related to HIV infection or to organ and marrow transplantation. With the introduction of combination anti-

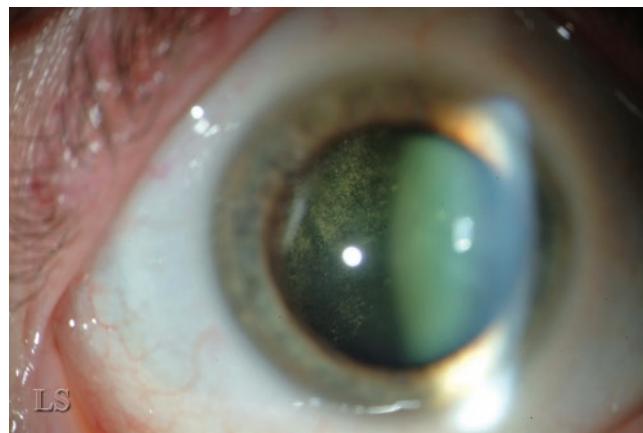


Figure 23-3 Intraocular large B-cell lymphoma on slit lamp examination.

retroviral therapy (cART), the incidence of PCNSL has decreased in HIV-infected persons. It appears, however, to be increasing in incidence in immunocompetent patients. In the latter group, the median age is 60 years, and it is discovered based on focal neurologic symptoms, personality changes, or symptoms of increased intracranial pressure. Ocular involvement can occur in 10% to 20% of patients and may be the sole site of disease at presentation (intraocular lymphoma). Concurrent leptomeningeal disease is found in 16% of patients through CSF analysis but occurs as the sole site in <5%. B symptoms, systemic symptoms of fever, night sweats, and weight loss, are extremely uncommon and should raise suspicions of systemic involvement.

Stereotactic-guided biopsy is the optimal method for diagnosing CNS lymphoma; gross total resection should be avoided. Steroids can interfere with pathologic diagnosis, and if they are started for neurologic symptoms, they should be withheld in patients with a presumptive radiologic diagnosis of CNS lymphoma to increase diagnostic biopsy yield. A contrast-enhanced MRI should be performed, along with lumbar puncture with CSF analysis. A slit-lamp examination should be performed to rule out concurrent ocular involvement. Staging should include full body PET/CT imaging, and, in men, testicular ultrasound because 4% to 12% of patients can have extraneuronal disease.

A prognostic scoring system has been developed in PCNSL, given the limitations of the Ann Arbor staging system and the IPI in this disease. The following five factors are associated with a poor prognosis: age older than 60 years; PS>2; elevated LDH; high CSF fluid protein concentration; and tumor location within the deep regions of the brain. Patients with 0, 1 to 4, or 5 of these factors have 2-year OS rates of 80%, 48%, or 15%, respectively.

The median survival after surgery alone is ~1–4 months. Whole-brain radiation is associated with a high response rate of 90%, but the median survival is only 12 months, and patients can develop significant cognitive dysfunction. CHOP has poor CNS penetration and should not be used in PCNSL. The exception is intravascular large B-cell lymphoma with CNS involvement because the mechanism of spread is likely different. Although there have been no randomized controlled studies to establish the best therapy, in retrospective analyses, outcomes are superior when high-dose methotrexate (HD-MTX) (3 to 8 g/m²) is incorporated into first-line regimens. With this approach, the 5-year OS is 30% to 40%. Some studies have added other CNS-penetrant chemotherapy drugs, such as cytarabine (ara-C). Rituximab therapy also appears to improve outcome. A phase 3 trial randomizing younger patients in a CR following HD-MTX to WBRT (45 Gy) or observation demonstrated an improvement in median PFS (18 months vs 12 months) but OS was similar, and toxicity was greater in patients who received radiation. For patients older than 60 years, the risks of neurotoxicity are considerable and manifests as dementia, ataxia, and incontinence, with a median time to risk-onset of approximately 1 year. Because of concerns of neurotoxicity even in younger patients, numerous studies are evaluating chemotherapy alone with CNS-penetrant drugs. The CALGB evaluated the combination of HD-MTX, temozolomide, and rituximab with consolidative HDC using ara-C and etoposide without WBRT; the 3-year PFS and OS were 50% and 67%, respectively. The international extranodal lymphoma study group (IELSG) conducted an important randomized trial, first randomizing patients to 1 of 3 induction arms: methotrexate and cytarabine (MA); methotrexate, cytarabine and rituximab (MAR); and methotrexate, cytarabine, thiotapec and rituximab (MATRix). For responding patients, a second randomization assigned patients to WBXRT versus HDC/ASCT. Results from the initial randomization showed that the MATRix combination resulted in the highest PFS and OS, followed by MAR, and then by MA. MATRix is therefore an appropriate standard of care in patients sufficiently fit to undergo this intensive chemotherapy approach.

The second randomization in the IELSG trial is based on increasing evidence of benefit for a thiotapec-based ASCT in CNS lymphomas. Several small phase 2 studies have evaluated upfront transplantation with cure rates ranging from 40% to 77% using a variety of lead-in chemotherapy and HDC regimens. In patients with relapsed or refractory primary CNS, HDC/ASCT is associated with a 2-year OS of 45%, a TRM of 16%, and severe neurotoxicity in 12%. The second randomization of the

aforementioned IELSG trial found identical 75% 2-year PFSs between HDC/ASCT and WBXRT but with significant neurotoxicity in the WBXRT arm, which therefore favors ASCT consolidation. Preliminary results of a GOELAMS study also comparing HDC/ASCT consolidation with WBXRT showed a PFS benefit favoring the transplantation arm, and a similar OS at 4 years. These data do support consideration of HDC/ASCT consolidation rather than WBXRT in young patients sufficiently fit to undergo transplantation.

For relapsed patients, methotrexate-based therapy is usually used again, particularly in those who have had a lengthy remission after initial therapy. Temozolamide alone or in combination with rituximab has shown an ORR of 26% and 53%, respectively, in relapsed and refractory patients. The combination of high-dose methotrexate, rituximab, and temozolamide (MRT) is well tolerated and associated with significant clinical activity in a small phase 2 study. CR was achieved in 14/18 (78%) patients at a median of 4 months. Three of 18 patients achieved a partial response (PR). At a median follow-up of 15.5 months from treatment initiation, 10/18 patients remain in CR and median PFS has not been reached. Novel biologically-directed therapies are also emerging in the management of relapsed/refractory PCNSL. The ABC subtype, which characterizes nearly all cases of primary CNS DLBCL, makes lenalidomide or ibrutinib appealing agents; both agents have demonstrated high response rates in small phase 2 studies. The 9p24.1 amplifications and PD-L1 expression make PD-1 inhibitors a potential option, and indeed small initial series have shown high and durable rates of remission. All three of these novel agents (lenalidomide, ibrutinib, and PD1 inhibitors) warrant ongoing study as single agents and in combination in the relapsed setting, as well as incorporation into frontline therapy.

Secondary CNS lymphoma

The rate of secondary involvement of CNS in aggressive lymphoma and lymphoblastic lymphoma, occurring in up to 30% of BL (see section Burkitt lymphoma in this chapter), varies by histology. In these highly aggressive lymphomas, CNS prophylaxis is routinely incorporated using intrathecal (IT) and systemic chemotherapy with or without cranial irradiation and has been shown to reduce the rate of CNS relapse and to prolong survival. Secondary CNS lymphoma may also be seen in DLBCL occurring in the brain parenchyma, leptomeningeal compartment, or both as an isolated event or with systemic relapse. Approximately 1% of patients with DLBCL have CNS involvement at diagnosis; the risk of subsequent CNS recurrence is approximately 4% but is increased in selected high-risk subgroups.

A number of extranodal sites have been associated with a higher risk of CNS relapse, including testis, kidney, and bone marrow (concordant). To create a robust risk model predictive of CNS recurrence risk, known as the CNS-IPI, the German High Grade Lymphoma Study group analyzed data on 2,164 patients treated with R-CHOP or R-CHOP-like therapy. The risk of CNS involvement was 3%, and adverse risk factors for CNS relapse on multivariable analysis were the 5 established IPI risk factors, plus renal or adrenal involvement. Using the total of these 6 risk factors present at diagnosis, three risk groups were created: low risk (0–1), intermediate risk (2–3), or high risk (4–6), with CNS relapse rates of 0.6%, 3.4%, and 10.2%, respectively. These data were validated in a 1,600-subject retrospective cohort from the British Columbia Cancer Agency and yielded similar results. Based on these data, patients with 4–6 CNS-IPI risk factors present at diagnosis would be classified as high risk for CNS recurrence and should be considered for CNS prophylaxis strategies.

Although these and other studies can effectively identify subgroups with a high risk for CNS disease, demonstrating a benefit for CNS prophylaxis has proven to be much more difficult in DLBCL. Furthermore, many of the studies evaluating CNS prophylaxis were published before the routine use of rituximab, which does appear to reduce risk, albeit to a modest degree. The RiCOVER-60 study evaluated 1,217 patients with aggressive lymphoma (81% DLBCL) and reported that 58 patients (4.8%) developed CNS relapse or progression with a median time of 8 months (1–39 months); the median survival from CNS relapse was only 3 months. Those patients who received rituximab had a lower risk of CNS relapse; however, the magnitude of difference was very small (3.6% vs 5.9%, $P=.043$). Other studies have confirmed that rituximab appears to reduce the risk of relapse, particularly in patients in a CR, suggesting the benefit, in part, may be due to better systemic disease control. The risk is not altogether eliminated, however, given the poor CNS penetration of rituximab. Modeled after BL and lymphoblastic lymphoma, intrathecal CNS prophylaxis often is administered to high-risk DLBCL patients, but the protective benefit is unknown, particularly because distribution within the leptomeningeal compartment is highly variable, and it offers no protection for the brain parenchyma which harbors the majority of DLBCL relapses in the CNS. Prophylactic use of HD-MTX (3.0 to 3.5 g/m²) with R-CHOP was evaluated retrospectively in 65 patients with high-risk DLBCL (elevated LDH, involvement of >1 extranodal sites, 4–5 Hollander criteria, high-risk location: bone marrow, testes, epidural, liver, adrenal, renal, orbit), and reported a low rate of CNS relapse (3%). Use of HD-MTX, however, is limited in elderly patients, particularly in those

with poor renal function. A similar strategy of systemic methotrexate prophylaxis is currently under evaluation in treatment of primary testicular DLBCL, a subset of DLBCL associated with a particularly high risk of CNS relapse in the study conducted by IELSG.

Despite the limitations and lack of evidence-based data to direct treatment, patients considered high-risk by the extranodal site involved or by the CNS-IPI model should be considered for CNS prophylaxis. Patients with any neurologic signs or symptoms should also be evaluated with diagnostic lumbar puncture including flow cytometry and brain MRI as appropriate. Our preferred method for CNS prophylaxis in eligible patients is systemic methotrexate 3.5 g/m² administered on day 15 of the 21-day R-CHOP-M cycle and usually administered with alternating cycles for a total of 3 methotrexate infusions, if tolerated. Intrathecal prophylaxis remains available for patients who are not considered candidates for systemic methotrexate therapy, such as patients who are very elderly or who have impaired renal function.

Burkitt lymphoma

BL is among the most aggressive of all human malignancies, with a rapid doubling time, acute onset, and progression of symptoms. Histologically, BL has a diffuse growth pattern of medium-size cells and a high mitotic rate; nearly 100% of cells are Ki-67 positive due to deregulated high-level expression of cMYC arising from reciprocal translocation with immunoglobulin-heavy (t8;14) or variant light-chain gene loci (t2;8 or t8;22) (Table 23-2). Additional mutations in the transcription factor that controls germinal center cell proliferation, *TCF3*, and its inhibitor, *ID3*, also cooperate with cMYC overexpression to drive proliferation. In conjunction with proliferation, there is also a high rate of cell death or apoptosis, and the dead cells are phagocytosed by histiocytes, which gives a “starry-sky” appearance at low power. The B cells are positive for CD19, CD20, BCL6, and CD10. BCL2 is usually negative, but rare weakly positive cases may be seen. Lack of TdT is critical to rule out ALL/lymphoblastic lymphoma. Recent studies have identified a subset of lymphomas that resemble BL by clinical course, morphology, immunophenotype, and gene expression but lack MYC rearrangements. This new provisional 2016 WHO entity has chromosome 11q alterations that appear to drive the Burkitt-like features (Table 23-3).

Originally described in its endemic form in African children presenting with jaw or facial masses, BL also occurs in sporadic form in the Western world, predominantly in children and young adults. It also is seen in HIV-infected patients. Nearly all endemic cases show evidence of EBV

infection and presence of the EBV genome, but such EBV infection is present in only a minority of sporadic cases.

Clinically, patients with BL frequently present with a bulky abdominal mass, B-symptoms and extranodal disease, including bone marrow involvement, is common (up to 70%). A leukemic phase can be seen, but pure acute leukemia is extremely rare. CNS dissemination, usually in the form of leptomeningeal involvement, may be present at diagnosis in up to 30% of patients; as a result, CNS chemoprophylaxis is integrated into the therapy for virtually all BL patients.

Therapy for BL must be instituted quickly because of the rapid clinical progression of the disease. Admission to hospital and tumor lysis precautions are essential and include vigorous hydration and allopurinol treatment with close monitoring of laboratory studies, including electrolytes and renal function. Recombinant uric acid oxidase (rasburicase) has been shown to be very effective in preventing uric acid nephropathy and its secondary metabolic complications. Multiple studies have shown that CHOP chemotherapy is inadequate for the treatment of BL, and intensified therapies result in higher cure rates. Multiagent combination chemotherapy, that includes high doses of alkylating agents and CNS prophylaxis, have improved the outcome for adults and children with the disease. Given the disease rarity, there are no randomized controlled treatment trials in adults comparing these approaches. Magrath et al, at the National Cancer Institute demonstrated a risk-adapted strategy that is useful for treatment stratification in both adults and children. Low-risk patients were those with a single extra-abdominal mass or completely resected abdominal disease and a normal LDH, and all other patients were considered high-risk. Low-risk patients received three cycles of cyclophosphamide, vincristine, doxorubicin, and methotrexate (CODOX-M), and high-risk patients received CODOX-M alternating with ifosfamide, etoposide, and cytarabine (IVAC) for a total of 4 cycles (i.e., 2 cycles each of CODOX-M and IVAC). All patients received intrathecal chemoprophylaxis with each cycle, and those with CNS disease at presentation received additional intrathecal therapy during the first 2 cycles. Approximately half of the patients were adults, and the 2-year EFS for all patients was 92%. Two other phase 2 studies have used the Magrath regimen with modifications. In a United Kingdom study, adult (age range, 16–60 years; median age, 26.5 years), non-HIV patients were treated with dose-modified CODOX-M (3 g/m^2) for 3 cycles if they were determined to be low risk (ie, normal LDH, PS of 0 or 1, Ann Arbor stage I or II, and no tumor mass $>10 \text{ cm}$), and all other patients were considered high risk and treated with alternating dose-modified CODOX-M/IVAC. The

2-year PFS for the patients with BL was 64%. A modified Magrath regimen was also studied in an older population of patients (median age, 47 years) with a reported 2-year EFS was 71%. Other therapeutic approaches have included the hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (HyperCVAD)/methotrexate-cytarabine regimen and ALL-type regimens. Retrospective analyses and a phase 3 trial evaluating the addition of rituximab to intensive chemotherapy for BL in adults demonstrated an improvement in PFS and establishes that rituximab should routinely be included in the treatment plan of these patients. Notably, the intensive regimens described above incur high rates of toxicity and are poorly tolerated by older adults. The results from 12 large treatment series (10 prospective and 2 retrospective) were combined to better determine outcome in patients with BL in patients older than 40 years. In total, 470 patients were identified, 183 of whom were older than 40 years. The median OS at 2 years with intensive short-duration chemotherapy in older patients was only 39% compared with 71% when all patients were considered, suggesting an unmet need in older BL patients. More recently, a phase 2 study at the National Cancer institute evaluated DA-EPOCH-R in 30 adult patients with BL. The treatment was well tolerated in older adults and produced a 5-year EFS of more than 90%. This approach has now been validated in a multicenter prospective phase 2 trial of 113 adults with BL treated at 22 centers in the US. At a median follow-up of 3 years, the EFS was 85.7%; treatment was equally effective in younger and older patients. Based on these data, DA-EPOCH-R can be considered an appropriate standard regimen for the treatment of BL and is preferred in older adults who do not tolerate intensive therapy well.

High-grade B-cell lymphoma, NOS

High-grade B-cell lymphoma, NOS, is a new diagnostic entity in the 2016 WHO classification that replaced the eliminated category of “B-cell lymphoma, unclassifiable, with features between DLBCL and BL.” Previously, B-cell lymphomas with morphologic and genetic features between DLBCL and BL, as well as a large proportion of DHLs (described above), were classified as “B-cell lymphoma, unclassifiable, with features between DLBCL and BL.” With the new classification scheme, DHLs are now classified as “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.” B-cell lymphomas with morphologic and genetic features between DLBCL and BL that lack the aforementioned gene rearrangements are now classified as “high-grade B-cell lymphoma, NOS.” Because this is a newly classified entity, the prognosis and

optimal management of these patients remains undefined. With the removal of the DHL patients from this category, the prognosis for the newly classified patients has likely improved. In the absence of data to guide therapy, most lymphoma specialists prefer more intensive strategies in these patients based on their high-risk histology, such as DA-EPOCH-R, which has been validated as effective in other high-grade B-cell lymphomas.

Immunodeficiency-associated lymphoproliferative disorders

Congenital or acquired immunodeficiency states are associated with an increased incidence of lymphoproliferative disorders. The WHO classification identifies four such categories: (i) primary immunodeficiency disorders, including Wiskott-Aldrich syndrome, ataxia-telangiectasia, common variable or severe combined immunodeficiency, X-linked lymphoproliferative disorder, Nijmegen breakage syndrome, hyper-IgM syndrome, and autoimmune lymphoproliferative syndrome; (ii) HIV infection; (iii) post-solid organ- or marrow-transplantation with iatrogenic immunosuppression; and (iv) methotrexate- or other iatrogenic-related immunosuppression for autoimmune disease. The lymphomas seen in these settings are heterogeneous and may include HL or, more commonly, aggressive NHL. Chédiak-Higashi syndrome also has been associated with an increased incidence of pseudolymphoma and true NHL.

Lymphoproliferative disorders associated with primary immune deficiencies (PIDs) most commonly are seen in pediatric patients and frequently are associated with EBV infection. Extranodal disease including the CNS is common. Lymphomas occurring in patients with PID do not differ morphologically compared with immunocompetent hosts. DLBCL is the most frequent histologic type, although T-cell lymphomas are more common in ataxia-telangiectasia. EBV-related lymphomatoid granulomatosis is associated with Wiskott-Aldrich syndrome. These malignancies respond poorly to standard therapy. Therapy depends on both the underlying disorder and the specific lymphoma subtype; allogeneic transplantation has been used successfully in some patients. Novel immunotherapeutic or pharmacologic strategies targeting EBV are being explored.

A newly recognized large B-cell lymphoma, that typically occurs in the setting of age-related or iatrogenic immunosuppression called EBV-positive mucocutaneous ulcer (Table 23-3), should be noted. Patients typically present with cutaneous or mucosal ulcers. The aggressive histologic features consist of large transformed EBV-positive B cells with Hodgkin-like features, which belies its indolent course with nearly all reported cases responding to reduction of immunosuppressive therapy.

HIV-associated lymphomas

HIV-associated lymphomas are most commonly DLBCL or BL, with rarer histologies including plasmablastic lymphoma and primary effusion lymphoma. Approximately two-thirds of DLBCL cases are EBV-associated. Outcomes for HIV-associated lymphomas were historically poor; however, since the advent of combination antiretroviral therapy (cART), outcomes in the modern era are similar to non-HIV lymphoma as long as the HIV is under good control and the CD4 count is over 200 cells/ μ L. Given the importance of optimal HIV control, cART is usually given concurrently with chemotherapy and in cooperation with the HIV specialist to avoid administration of antiretrovirals that can exacerbate chemotherapy toxicity.

Optimal chemotherapy and the role of rituximab with anthracycline combinations in HIV-associated DLBCL have been the subject of debate. One small randomized study conducted by the AIDS Malignancy Consortium (AMC 010) demonstrated no improvement in outcome comparing R-CHOP with CHOP and an increase in treatment-related infectious deaths. A subsequent analysis, however, indicated that the toxicity was higher in patients with a CD4 count <50. Furthermore, a phase 2 French study using R-CHOP in HIV-positive aggressive lymphomas (85% DLBCL) demonstrated a 2-year OS of 75% without an increase in life-threatening infections, which also may reflect the exclusion of poor-prognosis patients because patients could have no more than one of the following: CD4 < 100, PS >2, or prior AIDS. Thus, rituximab should be given to HIV patients if the CD4 count is >50, particularly given the strong evidence for improved survival in the HIV-negative setting. Concurrent administration of G-CSF is advised, given the high rate of infection in this population, and all patients should receive prophylaxis against *Pneumocystis jiroveci* infection. DA-EPOCH has been tested in HIV-aggressive lymphoma, the majority of which had DLBCL but with suspension of cART to avoid drug interactions. At 53 months, the PFS and OS were 60% and 73%, respectively. The AMC also tested EPOCH-R (AMC 034) in patients with HIV-positive, aggressive B-cell lymphomas with rituximab given concurrently or sequentially; the 2-year OS rates were 63% and 66%, respectively. The cART use was at the discretion of the treating physician but was used in the majority of patients. There was no greater risk of infection except in patients with a CD4 < 50. More recently, the NCI piloted a second-generation regimen short-course (SC)-EPOCH-RR (two doses of rituximab per cycle), with G-CSF support, in HIV-positive DLBCL patients with the goal of improving efficacy and reducing toxicity. Dose-dense rituximab was intended to enhance the chemo-

therapy and minimize the number of treatment cycles. A PET scan was performed after two cycles: if negative, only one more cycle was given; and if positive, two to three cycles were given. The 5-year PFS and OS were 84% and 64%, respectively. A pooled analysis of these two AMC trials with patients treated with R-CHOP or R-EPOCH suggested that patients receiving R-EPOCH had an improved EFS and OS after adjusting for the aaIPI and CD4 count. The TRM was greater in patients with CD4 counts <50 (37% vs 6%, $P=.01$) regardless of the regimen used. Despite the practice for many years at the NCI to suspend cART use during DA-EPOCH, modern cART regimens can safely be combined with chemoimmunotherapy; the combination is recommended by infectious-disease specialists, and should be considered the standard of care. Attention should be paid to certain classes of drugs that can cause drug-drug interactions, such as protease inhibitors, which may increase vincristine-associated toxicity. Among BL patients, both R-CODOX-M/R-IVAC and DA-EPOCH-R can be safely administered to HIV-BL patients. These patients should therefore be treated similarly to their HIV-negative counterparts.

Posttransplant lymphoproliferative disorders

Posttransplant lymphoproliferative disorders (PTLDs) occur as a consequence of immunosuppression in recipients of solid organ, bone-marrow, or stem-cell allografts. The risk is higher in solid-organ transplants that warrant a higher degree of immunosuppression (10%–25% in heart and lung transplants) than those that require a lower immune-suppression dosing (1%–5% kidney and liver transplants), but the most important risk factor for EBV-driven PTLD is pre-transplant EBV seronegativity. PTLDs are composed of a spectrum of disorders, ranging from EBV-positive infectious mononucleosis (early lesions) to polymorphic PTLDs, which most often are clonal, to full-blown monomorphic PTLDs that can be EBV-positive or EBV-negative and are further subdivided into B-cell lymphomas (common) with DLBCL being the most common, and T-cell lymphomas (rare); these are indistinguishable from their counterparts in immunocompetent hosts. HL-type PTLDs also can occur; however, indolent B-cell lymphomas arising in transplantation recipients are not among the PTLDs. EBV-negative PTLD has increased over the last decade and typically has a late onset (median time from transplantation to PTLD of 50–60 months vs 12 months in EBV-positive patients), a poorer response to therapy, and is more frequently monomorphic.

PTLDs have diverse clinical presentation depending on location. Extranodal involvement is common, particularly the gastrointestinal (GI) tract (~25%), lung,

skin, and bone marrow. Primary CNS lymphoma also can occur. The goal of treatment is to cure the lymphoma but also to preserve graft function. Although a significant minority (20–50%) of patients respond to a reduction in intensity of immunosuppressive drugs, most require additional systemic therapy, particularly for monomorphic or late PTLDs. Tolerance to chemotherapy is poor in PTLD patients, with treatment-related mortality reported to be as high as 31% in older series using CHOP chemotherapy. With historically poor tolerance to combination chemotherapy, single-agent rituximab has been explored in the first-line setting in PTLD. The ORR has ranged from 40% to 75%, and it is extremely well tolerated; however, remission duration may be short in many patients. In the first prospective phase 2 study, 43 PTLD patients who had failed to respond to a reduction in immunosuppression were treated with single-agent rituximab. The ORR was 44% at day 80 (CR 21%), and the 1-year OS was 67%. An updated analysis from this study evaluating 60 patients demonstrated an ORR of 59% (CR 42%), but the median PFS was only 6 months and the 2-year OS was 52%. Elevated LDH was predictive of disease progression as well as a shorter time from the date of transplant. Using a PTLD-adapted prognostic score incorporating age (>60 years), elevated LDH, and PS (>2), patients with a score of 0, 1, or 2/3 had 2-year OS estimates of 88%, 50%, and 0%, respectively, suggesting that single-agent rituximab may be suboptimal in high-risk groups. A subsequent phase II study, 152 patients with PTLD, who were treatment-naïve, were administered 4 weekly doses of rituximab, with subsequent therapy stratified based on CT scan response. Patients with a CR after rituximab alone received 4 additional doses of rituximab monotherapy at 21-day intervals, while patients without CR proceeded to 4 cycles of R-CHOP-21. Seventy percent of subjects achieved CR after rituximab monotherapy, with the remainder requiring R-CHOP. The 3-year PFS and OS in the entire population were 75% and 70%, respectively, suggesting that this sequential response-adapted treatment approach is a reasonable strategy and may avoid chemotherapy exposure in a significant proportion of patients. Reduced immunosuppression and single-agent rituximab are therefore reasonable first-line treatments in most patients with sequential therapy with R-CHOP reserved for those who do not achieve a CR after reduced immunosuppression and rituximab alone. For patients who present with very high-risk aggressive disease, R-CHOP can be considered frontline treatment with G-CSF support and inclusion of PJP prophylaxis.

Mantle cell lymphoma

MCL accounts for 6% of all NHLs and was characterized historically by poor outcomes and a short overall survival. But that was before treatments were developed specifically for this unique histology. Modern outcomes have markedly improved for younger and older patients alike, based on improved induction regimens and availability of targeted therapies at relapse.

MCL has distinctive clinical features including median age in the mid 60s, a striking male predominance, and a strong tendency to present with advanced-stage disease. Extramedullary involvement is common, including bone marrow and peripheral blood, plus a peculiar tendency to invade the GI tract, which may present as a distinctive syndrome of lymphomatous polyposis of the large bowel. Even patients without overt colonic polyposis frequently have subclinical GI epithelial invasion, which can be demonstrated on biopsy.

Cytologically, most MCLs consist of small lymphocytes with notched nuclei. The architectural pattern of the lymph node usually is diffuse but may show a vaguely nodular- or mantle-zone growth pattern. A spectrum of morphologic variants has been recognized which includes small cells, which are composed of small round lymphocytes and clumped chromatin, mimicking SLL/CLL, and a blastoid variant, which has a high mitotic rate and is clinically very aggressive. The immunophenotype of MCL is distinctive. Cases are typically CD5⁺, FMC7⁺, and CD43⁺ but CD10⁻ and CD23⁻ (Table 23-2). Some of the salient features that distinguish MCL from SLL or CLL are the expression of cyclin D1, SOX11, and FMC7 without CD23 expression (Table 23-2). Furthermore, MCL has a more intense IgM or IgD and CD20 expression than SLL/CLL. Virtually all MCLs carry the t(11;14)(q13;q32) on karyotypic analysis or by FISH. This reciprocal translocation juxtaposes the immunoglobulin heavy-chain locus and the cyclin D1 (*BCL1*) gene.

Biologic and clinical features have prognostic value in MCL. Cellular proliferation may be the most powerful predictor. cDNA microarray analysis has demonstrated that genes associated with cellular proliferation show striking variability among MCL cases, ranging from low to very high expression. Patients in the lowest quartile of expression have median survival times of 6–8 years, whereas patients in the highest expression quartile have survivals of <1 year. For clinical practice, Ki-67 staining can provide an estimate of proliferation. Three prognostic groups have been identified using cut points of <10% (best), 10% to 29% (intermediate), and >30% (worst). With regards to clinical factors, the IPI does not provide adequate prog-

nostic usefulness when applied to MCL, leading to the generation of an MCL-specific index. The MCL international prognostic index (MIPI) identified four clinical features, age, PS, LDH, and WBC, as independently associated with OS (Table 23-7). The MIPI score can separate patients into three risk groups and is quite valuable for characterizing patients in a clinical trial. Characterization is not always useful in clinical practice because older age and poor PS may classify a patient as “high risk,” but such a patient may not be a candidate for therapy intensification.

Of note, two types of clinically indolent MCL variants were recently recognized. One being in-situ mantle-cell neoplasia (Table 23-3), with the term neoplasia replacing lymphoma to emphasize the low rate of progression of this variant that is characterized by the presence of cyclin D1-positive cells in the mantle zones of otherwise normal follicles without evidence of nodal architectural disruption. Likewise, the second indolent MCL variant is a leukemic non-nodal MCL that is likely derived from a postgerminal-center B cell that usually lacks SOX11 expression. Patients with this variant typically present with peripheral blood lymphocytosis and splenomegaly without significant lymphadenopathy.

Management of newly diagnosed MCL

Initial therapy of MCL must be personalized to the patient, taking into account pathology, clinical presentation, age, and comorbidities. Patients with low-disease-burden asymptomatic MCL may safely be observed for a period of time, though most patients will require therapy. The indolent variants of MCL, which most commonly present with leukemic disease and splenomegaly with minimal adenopathy, are particularly good candidates for a period of observation, if asymptomatic. With patients in need of therapy, we typically divide them based on age (usually 65 or younger) and whether they are candidates for HDC/ASCT.

For younger patients with MCL, strategies incorporating rituximab, cytarabine, and HDC/ASCT consolidation have produced the best results with the longest PFS and OS. The Nordic Lymphoma Study Group phase 2 trial tested an intensive-induction immunochemotherapy with cycles of R-maxi-CHOP alternating with R-cytarabine, followed by in-vivo purge (with rituximab) and ASCT. The study was limited to patients younger than 65 years median age was 56 years. The ORR was 96%, and at 15 years of follow-up, the median PFS and OS were 8.5 years and 12.7 years, respectively. The European MCL Network has presented results of a large phase 3 randomized clinical trial with MCL patients <65 years. This trial compared the efficacy of six courses of R-CHOP

followed by HDC/ASCT vs alternating courses of R-CHOP/R-DHAP followed by a high-dose cytarabine containing HDC/ASCT. The study was designed to test the contribution of cytarabine in the management of younger MCL patients (median age 56 years). The 5-year PFS was significantly better in the cytarabine-containing arm (65% vs 40%). A recent prospective phase 3 trial from the French LYSA group administered 4 cycles of R-DHAP followed by HDC/ASCT in responding patients, who were then randomized to maintenance rituximab therapy vs no further therapy. The ORR and CRR after 4 courses of R-DHAP were 89% and 77%, respectively. Among randomized patients, the 4-year PFSs were 83% vs 64%, respectively, favoring maintenance rituximab. The 4-year OSs were also improved (89% vs 80%, respectively, $P=.04$), making maintenance rituximab the standard of care post HDC/ASCT in MCL.

Patients over the age of 60 have been evaluated in clinical trials which do not require HDC/ASCT. The European MCL Network conducted a trial for patients older than 60 years, who were assigned randomly to induction with R-CHOP or to the R-FC (rituximab, fludarabine, cyclophosphamide) regimen. Responding patients underwent a second randomization to maintenance therapy with rituximab (MR) or interferon- α (IFN α), each course given until progression. The median age of the 560 study participants was 70 years. Although response rates were similar between R-CHOP (86%) and R-FC (79%), the OS was significantly better in the R-CHOP arm (62% vs 47% at 4 years, $P=.005$). The inferior survival in the R-FC group was due to a combination of inferior disease control and increased death from infectious complications related to the immunosuppressive effects of fludarabine. Remission duration was significantly longer in the rituximab group than in the IFN group. At 4 years, 58% of the MR group remained in remission compared with 29% of the IFN group. Subgroup analysis indicated the benefit of MR was restricted to the R-CHOP-treated patients; the R-CHOP plus MR-treated patients experienced improved 4-year OS compared with R-CHOP- plus IFN-treated patients (87 vs 63%, $P=.005$), respectively. This trial indicates that R-CHOP followed by MR is a reasonable front-line approach for older MCL patients.

An additional phase 3 trial compared R-CHOP to an R-CHOP-like regimen (VR-CAP), where bortezomib replaced vincristine. The VR-CAP regimen was superior to R-CHOP for complete response rates (53% vs 42%), median PFS (24.7 months vs 14.4 months), and 4-year OS rate (64% vs 54%). The rates of neutropenia and thrombocytopenia were higher in the VR-CAP patients. Finally, the bendamustine-rituximab (BR) regimen also appears

to be a preferred alternative to R-CHOP. A large randomized trial compared BR with R-CHOP in patients with newly diagnosed indolent and MCL lymphoma. For the entire study population, BR was better tolerated than R-CHOP, with less alopecia, neutropenia, and infections. In the MCL patients ($n=93$), median age 70, BR was superior to R-CHOP for median PFS (35 months vs 22 months, $P=.006$). In a similarly designed trial was conducted in North America, MCL patients ($n=67$) comprised a subset of the study population. MCL patients assigned to BR were more likely to achieve complete remission than patients assigned to R-CHOP or R-CVP (50% vs 27%). Taken together, these studies suggest that the VR-CAP and BR regimens are better induction platforms than R-CHOP regimens in elderly patients with MCL, with BR being the best tolerated and most widely used. A small randomized trial evaluating MR after BR in MCL showed no improvement in this setting; therefore, BR without maintenance remains preferred when BR induction therapy is used.

Management of relapsed MCL

Younger patients relapsing after intensive therapies are candidates for allo SCT. The literature varies widely in the efficacy of this approach, but allo SCT does appear to have curative potential for a fraction of patients (25%-50%). A multicenter experience using a reduced-intensity conditioning (RIC) approach demonstrated 2-year EFS and OS rates of 50% and 53%, respectively. The 2-year transplant-related mortality rate was 32%, highlighting the high-risk/high-reward nature of allo SCT in relapsed MCL. For older patients, the BR regimen is highly active in relapsed MCL, with an ORR of 75% to 92% reported in two small studies. The proteasome inhibitor bortezomib is FDA-approved for relapsed MCL and has modest activity, with an ORR of 33% and a median PFS of 6 months. The mTOR inhibitor temsirolimus is European Union-approved for relapsed MCL, demonstrating an ORR of 22% and median PFS of 4.8 months in a pivotal study. Newer targeted therapies, however, are demonstrating improved clinical activity with decreased toxicity. The immunomodulatory agent lenalidomide is FDA-approved for recurrent MCL. In the EMERGE study ($n=134$), lenalidomide produced response rates of 28%. Although the median PFS was just 4 months, the median duration of response of 16.6 months indicated that responders can experience a durable benefit. Lenalidomide, which potentiates immune-effector cells, appears to be even more active when combined with rituximab. A phase 1/2 trial in relapsed MCL ($n=52$) reported an ORR of 57% and a median PFS of 11.1 months. Most promising of the new

agents are the Bruton tyrosine kinase (BTK) inhibitors, which interfere with signaling through the B-cell receptor pathway. In a single arm phase 2 trial ($n=111$) in relapsed/refractory MCL, the BTK inhibitor ibrutinib produced an ORR of 68%, CRR of 21%, and median PFS of 13.9 months. Ibrutinib was FDA-approved for patients with recurrent MCL in late 2013. A second generation BTK inhibitor, acalabrutinib has also been FDA-approved for relapsed/refractory MCL based on a 124-patient multi-center phase 2 study showing an ORR of 81% with CRR of 40% and a 12-month PFS of 67%. BTK inhibitors and lenalidomide are currently being explored in addition to up-front therapy and may ultimately decrease our reliance on intensive chemotherapy and stem-cell transplantation.

Peripheral T-cell lymphomas

PTCLs represent 10% to 15% of all NHLs in Western populations and are a heterogeneous group of mature T-cell neoplasms arising from postthymic T cells at various stages of differentiation. NK-cell lymphomas are included in this group because of the close relationship between these two cell types. The importance of the T-cell phenotype and the impact on prognosis are now well established but are relatively recent advances. A large retrospective study, the International T-Cell Lymphoma Project (ITLP), collected 1,153 cases of PTCLs from 22 centers from around the world and highlighted the geographic, clinicopathologic, and prognostic differences of this diverse group of diseases. There is a range of diseases among T- and NK-cell neoplasms, with most diseases behaving aggressively; however, a minority have a favorable prognosis or an indolent course (Table 23-3).

Indolent PTCLs

Mycosis fungoides and Sézary syndrome

In contrast to nodal NHLs, which are mostly B-cell derived, ~75% of primary cutaneous lymphomas have a T-cell phenotype and two-thirds are mycosis fungoides (MF) or Sézary syndrome (SS). MF is an epidermotropic, primary cutaneous T-cell lymphoma and represents the most common of all primary cutaneous lymphomas (50%). MF usually has an indolent course, but, like indolent B-cell lymphomas, it is considered incurable using conventional therapies. MF is limited to the skin in its early phases and appears as plaques or patches; but, with time, it evolves to diffuse erythroderma or cutaneous nodules or tumors, usually with associated adenopathy. The early-stage lesions appear characteristically in a bathing suit distribution and are often pruritic in nature. Extracutaneous disease can occur in advanced stages and may indicate histologic transformation. The histology varies with stage of the disease,

but epidermotropism is seen with typical plaques and intradermal collections of so-called Pautrier microabscesses. The T-cells are CD4⁺/CD8⁻, often with aberrant loss of one or more of the T-cell antigens CD2, CD3, CD5, and CD7. Progression to nodal disease, organ infiltration, and circulating clonal T-cells (SS) represents the advanced stage of the disease. A unique clinical staging system has been proposed by the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC) for MF and SS. The extent of cutaneous and extracutaneous disease is the most important prognostic factor in MF, with a 10-year disease-specific survival ranging from 97% to 98% for patients with limited patch/plaque disease (<10% of skin surface; stage I) to 20% for patients with lymph-node involvement.

SS is a distinct disorder characterized by erythroderma, generalized lymphadenopathy, and the presence of Sézary cells in the skin, lymph nodes, and peripheral blood. It is associated with an aggressive course with a 5-year OS rate of 20% to 30% with lower rates seen with high Sézary cell counts.

Because MF is incurable and the use of early therapy does not affect survival, a nonaggressive approach is recommended. Patients with stage IA disease may be managed expectantly with careful surveillance. If treatment is needed, topical steroids or topical nitrogen mustard, electron-beam radiotherapy, or cutaneous photochemotherapy with oral psoralen plus ultraviolet A (PUVA) typically are employed. Phototherapy with PUVA or ultraviolet B (UVB) is recommended for more widespread disease. Low-dose radiotherapy can be helpful to improve symptoms and cosmesis. Patients with progressive disease and those with systemic dissemination may be appropriately treated with methotrexate or corticosteroids, although responses are usually brief.

Combination chemotherapy regimens are not particularly effective and provide only transient responses. Single-agent treatments are preferred, particularly with slowly progressive disease, because of a high risk of myelosuppression and infection and only modest response durations seen with combination chemotherapy. Gemcitabine (ORR 48%-75%), pentostatin (ORR 28%-71%), and liposomal doxorubicin (ORR 56%-88%) have single-agent activity. Alternatively, IFNa, bexarotene, vorinostat, romidepsin, and brentuximab vedotin all have efficacy in advanced-stage MF and SS. Brentuximab vedotin is preferred in CD30-positive cases based on the international phase 3 ALCANZA trial where 131 patients with CD30-positive relapsed/refractory MF or CTCL were randomized between the anti-CD30 antibody drug conjugate brentuximab vedotin, or the in-

vestigator's choice of oral methotrexate or oral bexarotene. Patients treated with brentuximab vedotin had significant improvement in the primary endpoint of objective response lasting at least 4 months (56.3% vs 12.5%), resulting in FDA-approval for brentuximab vedotin in this indication.

Bexarotene is an oral retinoid and is FDA-approved for cutaneous T-cell lymphoma (CTCL). In a multicenter trial of 94 patients with advanced stage MF/SS, the ORR was 45% but with only 2% CRs. The common toxicities are hypertriglyceridemia (82%) and central hypothyroidism (29%). The histone deacetylase (HDAC) inhibitors, vorinostat and romidepsin, are both approved for the treatment of CTCLs. Vorinostat is available orally and has an ORR of ~30% and a median duration of response (DOR) of ~6 months. A phase 2 trial with romidepsin demonstrated an ORR of 35% (CR 6%) with a median DOR of 15 months in one study and 11 months in another. Side effects that are common with histone deacetylase (HDAC) inhibitors are fatigue, nausea, vomiting, neutropenia, and thrombocytopenia. Prolonged QT syndrome also can occur, and thus electrolytes should be monitored closely, and an electrocardiogram should be performed in high-risk patients during therapy. Alemtuzumab, the humanized monoclonal antibody targeting CD52, also has been used in MF and SS with some success; however, patients are at high risk of opportunistic infections. Studies evaluating low-dose alemtuzumab (10 mg thrice weekly) have been similarly effective with reduced toxicity, and should be preferred. Small studies also report single-agent activity for lenalidomide (ORR 28%) and low dose pralatrexate given at 15 mg/m² for 3 of every 4 weeks (ORR 45%).

Allogeneic transplantation has been explored in selected cases of MF and SS. The European Group for Blood and Marrow Transplantation recently reported a multi-institutional retrospective study evaluating allo SCT (myeloablative and RIC) in 60 patients with MF ($n=36$) or SS ($n=24$). Almost half had refractory disease at the time of allo SCT; the median number of prior regimens was four. With a median follow-up of 3 years, the 3-year PFS and OS were 34% and 53%, respectively, with higher survival rates observed in the RIC group (3-year PFS 52% vs 29%, $P=.006$).

Large-cell transformation in MF is defined as large cells in >25% of the infiltrate or as cells forming microscopic nodules. The incidence ranges from 8% to 39% and typically is associated with a poor prognosis, but there have been some long-term survivors. One study evaluated 100 cases of transformed MF; the median survival was 2 years with a 5-year OS and a disease-specific survival (DSS) of 33% and 38%, respectively, compared to MF patients without transformation. The factors associated with a poor

DSS were CD30-negative status, folliculotropic MF, generalized skin lesions, and extracutaneous transformation. Those cases with zero factors had a 2-year DSS of 83% compared with 14% to 33% in patients with three or four factors. The optimal management is unclear, but for young patients, systemic chemotherapy should be used and autologous or allogeneic transplantation should be considered particularly with high-risk disease. Consolidative radiation may be an option in local transformations.

Primary cutaneous ALCL

Primary cutaneous ALCL (C-ALCL) is part of a spectrum of diseases belonging to the category of primary cutaneous CD30⁺ T-cell lymphoproliferative disorders that also includes lymphomatoid papulosis and "borderline" cases that have overlapping features of both disorders. C-ALCL is the second most common type of CTCL. Patients are typically older males (median age 60 years), presenting with a solitary nodule with multifocal disease occurring in only 20% of patients. Partial or complete spontaneous regression occurs in ~25% of cases. C-ALCL must be distinguished from systemic ALCL with secondary cutaneous involvement through staging procedures.

The outcome is very favorable with a 10-year DSS of 95%. It is notable that patients with localized C-ALCL with one draining lymph node involved have a similarly good prognosis. For localized C-ALCL, radiation is the preferred therapy. Progression to systemic involvement can occur in a minority of cases. For more advanced-stage cases, the best management is unclear. An argument can be made to treat minimally symptomatic patients conservatively with palliative dose radiotherapy just to the few most prominent lesions, but for patients where systemic therapy is required, brentuximab vedotin is preferred based on the aforementioned data for this agent in CD30⁺ CTCL.

T-cell large granular lymphocytic leukemia and chronic lymphoproliferative disorder of NK cells

T-cell large granular lymphocytic leukemia (T-LGL) is defined by a persistent (>6 months) increase in the number of peripheral-blood large granular lymphocyte cells without an identifiable cause. The lymphocytosis is usually between 2×10^9 and $20 \times 10^9/L$. The malignant T-LGL cells are positive for CD3 and CD8, and CD57/CD16 are expressed in most cases, but CD56 is negative. It may arise de novo or in the context of rheumatoid arthritis or other autoimmune disorder. T-LGL must be distinguished from reactive LGL populations which may be seen in the setting of chronic viral infections or autoimmune conditions. Assessment of clonality with T-cell receptor PCR is often helpful

in establishing the diagnosis. Most cases have an indolent clinical course, and T-LGL is usually not considered a life-threatening disease; however, rare cases with an aggressive course have been described. Chronic lymphoproliferative disorder of NK cells (CLPD-NK) have similar clinic features and indolent course, but the neoplastic cells have an NK cell immunophenotype with expression of CD16 and CD56, variable expression of CD2, CD5, and CD7, and lack of surface CD3. STAT3 mutations are found in about 30% of both T-LGL and CLPD-NK. Of note, T-LGL and CLPD-NK should be distinguished from aggressive NK-cell leukemia, which have a fulminant aggressive course (see the section Aggressive NK-cell leukemia). In T-LGL and CLPD-NK, moderate splenomegaly is the most common clinical finding, and lymphadenopathy is rare. Severe neutropenia with or without anemia is common, and pancytopenia may be seen. A variety of autoimmune disorders, including hemolytic anemia, thrombocytopenia, and pure red blood cell aplasia, also may occur. If treatment is required for cytopenias, immunomodulatory agents, such as low-dose methotrexate, cyclophosphamide, and cyclosporine A, are often effective, and corticosteroids can provide a useful adjunct. Responses can take up to 4 months, and longer therapy often is needed to maintain the response. Weekly low-dose oral methotrexate is most commonly used as initial therapy, though oral cyclophosphamide at a dose of 50 to 100 mg by mouth daily has anecdotally appeared to be more effective in anemia-predominant disease. Purine analogs have been used in highly refractory patients. Splenectomy may be useful in selected cases with an accompanying splenomegaly, refractory cytopenias, or autoimmune hemolytic anemia or thrombocytopenia. The anti-CD52 monoclonal antibody alemtuzumab can be used in select cases.

Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract

Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract is a clonal proliferation typically involving CD8-positive T cells that infiltrate the lamina propria of multiple sites in the small intestine and colon. Patients typically present with abdominal pain, dyspepsia, diarrhea, and weight loss. Biopsies demonstrate a lymphoid infiltrate in the lamina propria that shows little histologic evidence of epithelial invasion, and, accordingly, patients generally have an indolent relapsing clinical course. Response to chemotherapy is poor, but patients have prolonged survival with persistent disease.

Primary cutaneous acral CD8⁺ T-cell lymphoma

Primary cutaneous acral CD8⁺ T-cell lymphoma is a rare cutaneous lymphoma that typically occurs at acral sites,

such as the ear, nose, or soles of the feet as an isolated papule or nodule with a history of slow growth. Histologically, there is a dermal proliferation of intermediate-sized atypical CD8⁺T cells that lacks aggressive features, such as angiodestruction and necrosis, and spares the epidermis. Local excision or radiotherapy typically leads to complete remission.

Aggressive PTCLs

Adult T-cell leukemia/lymphoma

Adult T-cell lymphoma/leukemia (ATLL) is caused by infection with HTLV-1 and occurs in areas of endemic infection (eg, the Caribbean basin and southwestern Japan). The cumulative incidence of ATLL among HTLV-1 carriers is 2.5% in Japan. The virus can be transmitted in breast milk and blood products. The malignant cells have a distinct cloverleaf appearance and are CD7⁻, and most are CD4⁺/CD8⁻ and CD25⁺. The following clinical variants have been recognized: (i) acute type with a rapidly progressive clinical course including bone-marrow and peripheral-blood involvement, hypercalcemia with or without lytic bone lesions, skin rash, generalized lymphadenopathy, hepatosplenomegaly, and pulmonary infiltrates; (ii) lymphoma type with prominent adenopathy but lacking peripheral blood involvement but also associated with an aggressive course; (iii) chronic type with lymphocytosis and occasionally associated with lymphadenopathy, hepatosplenomegaly, and cutaneous lesions but having an indolent course; and (iv) smoldering type with <5% circulating neoplastic cells, skin involvement, and prolonged survival. The chronic and smoldering forms can progress to the acute form after a variable length of time. In the ITLP, 126 patients (9.6% of all PTCLs) were identified with the acute (13%) or lymphoma-type (87%) ATLL. Opportunistic infections are common, and *Strongyloides* serology is recommended before starting therapy.

Survival times in the acute and lymphomatous variants are ~6 and ~10 months, respectively. The median survival for the chronic form is 2 years. The 4-year OS for the acute, lymphoma, chronic, and smoldering types has been reported to be 5%, 5.7%, 27%, and 63%, respectively. Asymptomatic patients with the smoldering or chronic type ATLL can be monitored closely. For young, fit patients with the acute and lymphoma subtypes, the intensive chemotherapy regimen incorporating VCAP (vincristine, cyclophosphamide, doxorubicin and prednisolone)/AMP (doxorubicin, ranimustine, prednisolone)/VECP (vindesine, etoposide, carboplatin, prednisolone) may be considered. The Japan Clinical Oncology Group (JCOG) reported a phase 3 trial comparing the dose-intensive regimen VCAP/AMP/VECP versus CHOP-14 alone that showed a more favorable CR rate (40% vs 25%, $P=0.02$) and 3-year OS (24% vs 13%) that was significant after adjusting for prognostic

factors but only for the one-sided *P*-value (*P*=0.028). The median survival for the intensive regimen was just over 1 year, but toxicity was high (grade 4 neutropenia in 98% and grade 3/4 infections in 32%). Thus, this regimen should be used only in carefully selected patients, particularly with the lymphoma subtype. Relapse rates remain high, and relapsed patients should be considered for transplantation.

A number of phase 2 studies evaluating the use of the antiretroviral zidovudine (AZT) and IFN in untreated patients have found response rates up to 92% and a median OS of 11 months. For patients with the leukemia subtype, these results are superior to what is achieved with combination chemotherapy, though the benefit appears minimal in the lymphoma subtype. For patients with the chronic and smoldering types, a meta-analysis demonstrated 100% OS after 10 years with this approach.

Chemokine receptor 4 (CCR4) is expressed in ~90% of cases of ATL. Mogamulizumab (KW-0761) is a humanized monoclonal antibody targeting CCR4; a phase 2 study demonstrated an ORR of 50%, including eight CRs, in 27 treated patients. The median PFS and OS were 5.2 months and 13.7 months, respectively. The most common side effects were lymphopenia (96%), neutropenia (52%), thrombocytopenia (52%), infusion reaction (89%), and skin rashes (63%).

PTCL, not otherwise specified; systemic anaplastic large cell lymphoma; and angioimmunoblastic T-cell lymphoma

PTCL-NOS, systemic anaplastic large-cell lymphoma (ALCL), and angioimmunoblastic T-cell lymphoma (AITL) are the most common subtypes of PTCL encountered in North America and represent 66% of all PTCL cases.

PTCL-NOS

PTCL-NOS is the most common subgroup of PTCLs, accounting for up to 30% of cases worldwide. PTCL-NOS is the default PTCL category for any mature T-cell neoplasm that does not fit into any of the specified categories in the WHO classification. Patients typically present with advanced-stage disease, and the 5-year OS is 20% to 30% in most series. The morphologic spectrum of PTCL-NOS is wide, including the histiocyte-rich lymphoepithelioid, or Lennert, lymphoma. Typically, the neoplastic cells are CD4⁺/CD8; CD5 and CD7 frequently are lost, and ~30% are CD30⁺.

Treatment approaches in PTCL have paralleled those for DLBCL; as a result, CHOP-like therapy is routinely employed as frontline therapy. The DSHNHL group retrospectively analyzed the outcome of PTCL patients (*n*=331) that had been enrolled in phase 2 or phase 3 aggressive

lymphoma studies and evaluated the impact of etoposide. In patients younger than 60 years with a normal LDH, EFS was extended with etoposide (*P*=.003), whereas OS did not improve significantly (*P*=.176). The addition of etoposide appeared to have the greatest impact in the favorable group of patients with ALK-positive ALCL (3-year EFS 91% vs 82%, *P*=.012). In patients with PTCL-NOS, ALK-negative ALCL, and AITL, there was a trend toward improved 3-year EFS (61% vs 48%; *P*=.057), with no OS difference observed; however, patient numbers were small. On the basis of these data, CHOEP may be considered as initial therapy in younger patients. For sufficiently young and fit patients, upfront consolidation with HDC/ASCT is generally considered (see Transplantation in PTCL below).

Newer chemotherapies and targeted agents are available for relapsed disease. Pralatrexate is a novel folate analogue that has enhanced uptake and cellular retention compared with MTX. Early studies suggested a sensitivity of TCLs over BCLs. The phase 2 PROPEL study evaluated pralatrexate (with vitamin B₁₂ and folate) in relapsed/refractory PTCLs and demonstrated an ORR 29% (CR 11%), a median PFS of 3.5 months and a median duration of response (DOR) of 10.5 months. The main toxicities were mucositis, thrombocytopenia, and neutropenia. These results led to FDA approval of pralatrexate in September 2009 for the treatment of relapsed/refractory PTCL. Studies combining pralatrexate with other agents in the upfront and relapsed settings are ongoing.

Romidepsin is an HDAC-inhibitor that has been evaluated in CTCLs and PTCLs. A phase 2B registration study evaluated romidepsin in 130 patients with relapsed or refractory PTCL. The ORR was 25% (CR 15%), median DOR was 17 months, and median PFS was 4 months, leading to FDA approval in 2011. Side effects were as previously described in the CTCL studies. A phase 1b study is ongoing combining CHOP with romidepsin for the primary treatment of PTCL.

Belinostat is another HDAC-inhibitor that has demonstrated responses in relapsed or refractory PTCL in a phase 2 trial. Belinostat was granted approval by the FDA for the treatment of patients with PTCL who have received at least one prior therapy. A phase 2 trial (BELIEF trial) of belinostat in 120 patients with PTCL reported overall and complete remission rates of 26% and 11%, respectively, with a median DOR of 13 months.

CD30 is expressed uniformly in ALCL but is also highly restricted, making it an attractive target in this disease. Studies with the nascent anti-CD30⁺ in relapsed systemic ALCL were largely disappointing, however. Therefore, an antibody-drug conjugate (ADC), brentuximab vedotin, was

developed to enhance tumor activity. The ADC conjugates the CD30 monoclonal antibody to the microtubule inhibitor, monomethyl auristatin E (MMAE), by an enzyme-cleavable dipeptide linker. Following binding to CD30⁺ and uptake into the cell, MMAE is released and interferes with tubulin formation. A phase 2 study, recently reported in relapsed or refractory systemic ALCL, demonstrated an ORR of 86% (CR 57%), median DOR of 12.6 months, and a median PFS of 13.3 months, which also prompted FDA approval of brentuximab vedotin for ALCL in 2011. The main side effect of brentuximab vedotin is peripheral neuropathy. Studies are ongoing evaluating brentuximab vedotin in the up-front setting with CHOP, omitting vincristine because of overlapping toxicity.

Angioimmunoblastic T-cell lymphoma and nodal lymphomas of T follicular helper (TFH) cell origin

AITL is a well-defined, distinct PTCL subtype with unique pathobiologic features. Key morphologic findings of AITL include an expanded CD21⁺ follicular dendritic-cell network and prominent arborizing high-endothelial venules (HEV). The neoplastic cells in AITL are mature CD4⁺/CD8⁻ T-cells, expressing most pan-T-cell antigens. EBV-positive B cells are seen in most cases, and EBV-positive DLBCL has been reported. It appears that the cell of origin is the follicular helper T-cell with T-cells CD10⁺, BCL6⁺, and CXCL13⁺, and derivation also is supported by gene-expression profiling studies. Sequencing studies have shown this PTCL subtype to be enriched for mutations of *TET2*, *IDH2*, *DNMT3A*, *RHOA*, and *CD28*.

Patients are typically in their sixth or seventh decade and have advanced-stage disease, often with B-symptoms and hepatosplenomegaly. AITL was originally believed to be a form of immune dysregulation, with polyclonal gammopathy and other hematologic abnormalities (Coombs-positive hemolytic anemia) reflecting B-cell dysregulation. Opportunistic infections can occur because of the underlying immunodeficiency.

Survival is similar to that in PTCL-NOS (5 year ~30%); however, a small proportion of patients may have a more indolent course. CHOP or CHOEP is typically used as primary therapy, and, although the response rate is high, relapse is common and infectious complications are problematic. GELA evaluated AITL patients enrolled in different therapeutic protocols and found no improvement of survival with any therapy, including HDC/ASCT. Because of poor outcomes using conventional therapy, immuno-modulatory agents, including cyclosporine, lenalidomide,

thalidomide, and interferon, also have been explored. A retrospective study evaluating cyclosporine in relapsed or refractory AITL demonstrated an ORR of 67% and a median DOR of 13 months. Among patients with relapsed disease, the HDAC inhibitors appear to have improved activity in AITL relative to other PTCL subtypes, making these agents appealing for patients failing frontline chemotherapy. Similarly, brentuximab vedotin has produced encouraging response rates in relapsed AITL, where the infiltrating B immunoblasts are usually CD30⁺.

Follicular T-cell lymphoma and nodal PTCL with TFH phenotype are also distinct nodal T-cell lymphomas derived from the same TFH cell as AITL. They share recurrent genetic abnormalities with AITL, including *TET2*, *IDH2*, *DNMT3A*, *RHOA*, and *CD28* mutations as well as t(5;9) *ITK-SYK* fusion. The clinical course of these rarer variants is not yet well characterized, but they appear to have an aggressive clinical course similar to AITL.

Systemic anaplastic large-cell lymphoma

ALCL is composed of large CD30⁺ anaplastic cells with a predilection for a sinusoidal and cohesive growth pattern. In the WHO classification, systemic ALCL is separated from primary cutaneous ALCL. Systemic ALCL cases are divided into two groups: ALK-positive and ALK-negative. (Table 23-3). Cases of ALK-positive ALCL are associated with a characteristic chromosomal translocation, t(2;5) (p23;q35), resulting in a fusion gene, *NPM-ALK*, encoding a chimeric protein with tyrosine kinase activity. With the availability of antibodies to the ALK protein, ALK expression can be demonstrated in 60% to 85% of all systemic ALCL, with higher frequencies seen in the pediatric and young adult age-groups.

ALK-positive ALCL. Morphologically, ALK-positive ALCL has pathognomonic “hallmark cells” recognized by their eccentric, horseshoe, or kidney-shaped nuclei. In addition to strong expression of CD30, ALK-positive ALCL is usually positive for epithelial membrane antigen (EMA) and cytotoxic markers (TIA1, granzyme B, and perforin). Several studies have established that patients with ALK-positive ALCL have a more favorable prognosis with anthracycline-based chemotherapy than patients who have ALK-negative ALCL and other PTCLs, as well as DLBCL, at least in the prerituximab treatment era. The improved outcome, at least in part, is related to the young age and low risk features often present at presentation. The ITLP confirmed the superior outcome of ALK-positive ALCL (5-year FFS, 60%; 5-year OS, 60%) compared with ALK-negative ALCL (5-year FFS, 36%; 5-year OS, 49%). If the

comparison is confined to patients younger than 40 years, however, there was no difference in survival. Similar findings were reported from a retrospective analysis of patients with ALCL enrolled on GELA studies, which reported that, in patients younger than 40 years, there was no impact of ALK status on PFS or OS.

Given the favorable outcome with anthracycline-based chemotherapy, CHOP-like therapy is considered the standard therapy for ALK-positive ALCL. A subset analysis of ALK-positive ALCL patients treated in prospective studies from the German High Grade Lymphoma Study Group has identified a particularly favorable outcome among patients treated with CHOEP (3 year EFS, 92%). More recently, a randomized phase 3 trial evaluated the upfront addition of BV (brentuximab vedotin) to CHP (cyclophosphamide, doxorubicin and prednisone), compared to standard CHOP, in CD30⁺T-cell lymphomas (70% were ALCL). 452 patients were randomized, and the study found an improved PFS favoring the BV-CHP arm (hazard ratio 0.71, p=0.01). Overall survival was similarly improved among BV-CHP treated patients (hazard ratio 0.66, p=0.024). Based on these data, BV-CHP can now be considered standard frontline therapy for ALK+ or ALK- ALCL.

Crizotinib and other small molecule inhibitors of the ALK tyrosine kinase, FDA-approved for treatment of ALK-positive non-small-cell lung cancer, have also demonstrated remarkable clinical activity in patients with multiply relapsed ALK-positive anaplastic large-cell lymphoma (ALCL) and may be considered in patients with disease that has been refractory to both chemotherapy and brentuximab vedotin.

ALK-negative ALCL. Patients with ALK-negative ALCL tend to be older at presentation; the clinical presentation is similar to PTCL-NOS, but sites of extranodal disease may vary. Histologically, ALK-negative ALCL is not reproducibly distinguished from the so-called common pattern of ALK-positive ALCL except that it lacks the ALK protein. ALK-negative ALCL has been difficult to define, in part, due to a lack of uniformly applied diagnostic criteria across studies. Previously, it was argued that ALK-negative ALCL had an outcome similar to that of PTCL-NOS and the two should be grouped together. In recent years, it has become clear that they differ not only pathologically and genetically but also prognostically. The ITLP compared the outcome of ALK-negative ALCL with PTCL-NOS and established that ALK-negative ALCL had a more favorable 5-year FFS (36% vs 20%, P=.012) and OS (49% vs 32%, P=.032). Gene-

expression studies have shown that ALK-negative ALCL has a signature distinctly different from PTCL-NOS and similar to that of ALK-positive ALCL. A subset of ALK-negative ALCL cases carry *DUSP22-IRF4* rearrangements and appear to have superior outcomes, similar to that of ALK-positive ALCL, while another subset carrying *TP63* rearrangements have poor outcomes. These data confirm that ALK-negative ALCL should be considered distinct from both ALK-positive ALCL and PTCL-NOS. Although the survival for ALK-negative ALCL is more favorable than for PTCL-NOS, it is still poorer than for ALK-positive patients, except in patients carrying the *DUSP22-IRF4* rearrangement. Initial therapy is with the BV-CHP regimen based on the aforementioned randomized trial showing superiority over CHOP. Upfront consolidation with HDC/ASCT is generally considered for ALK-negative patients, particularly those lacking the *DUSP22-IRF4* rearrangement (see the section on transplantation below). Brentuximab vedotin is highly effective in the relapsed/refractory setting, if it had not been incorporated with frontline therapy.

Breast-implant-associated ALCL. ALCL associated with implants typically presents as an unexplained seroma or capsule thickening. The lymphoma typically involves the capsule only, without invasion of the breast tissue or formation of discrete mass lesions. Almost all cases are localized. The tumor cells are CD30⁺ and ALK negative. The neoplastic cells float in the effusion fluid or become embedded tissue; importantly, however, breast parenchyma usually is not involved, and the ALCL cells infiltrate the cavity containing the implant rather than the breast tissue directly. Breast-implant-associated ALCL has been associated with both silicone and saline implants, but, importantly, it occurs almost exclusively in implants with a textured, as opposed to a smooth, surface. A total capsulectomy should be performed, and, because bilateral cases have been reported, removal of the uninvolved breast implant is generally considered. The growing body of literature supports that ALK-negative ALCL in this setting appears to have an indolent clinical course with a favorable prognosis, and most patients can be observed following removal of the implant and capsule and will not require adjuvant therapy. Recent reports suggest similar survival rates compared with those who received chemotherapy or radiation; however, rare aggressive cases have been reported where chemotherapy may be required. Cases that have identified a distinct breast mass may be better classified as a typical systemic ALK-negative ALCL and may be treated accordingly.

Extranodal NK-/T-cell lymphoma, nasal type

Extranodal NK-/T-cell lymphoma, nasal type, display great variation in racial and geographic distribution, with the majority of cases occurring in Asia. Patients are typically males aged 40 to 50 years. The tumor cells show angioinvasion and prominent necrosis. The designation NK/T is used to reflect the fact that, although most cells are NK-cell derived (CD2⁺, CD56⁺, CD3 [cytoplasmic]⁺, EBV⁺), rare cases with identical clinical and cytologic features exhibit an EBV-positive or CD56⁻, cytotoxic T-cell marker positive (TIA1, perforin, and granzyme B). Circulating EBV in the peripheral blood can often be detected, providing another method of disease monitoring. Most cases remain localized but may be extensively locally invasive, with <20% of patients presenting with advanced-stage disease. Despite the predominant nasal location, spread to the CSF is uncommon. Most occur in the nasal region, but identical tumors also can occur at extranasal sites, such as skin, soft tissue, GI tract, and testis (ie, extranasal). It appears that cases involving extranasal regions may have a more aggressive course. From the ITLP, the 5-year OS for stage I/II NK-/T-cell lymphomas were ~50% and 15% for nasal and extranasal sites, respectively, and the corresponding estimates for stage III/IV patients were 30% and <10%. The IPI does not stratify patients well because most have localized disease and often with good PS. A Korean index, using B symptoms, stage (I/II vs III/IV), regional lymph nodes, LDH, and PS, appears to be more useful in prognostication, particularly for the low- and low-intermediate IPI cases and may help to guide treatment decisions. Patients fall into four risk groups with widely disparate outcomes: group 1: no RF, 5-year OS ~81%; group 2: 1 RF, 5-year OS ~64%; group 3: 2 RF, 5-year OS ~34%; and group 4: 3 or 4 RF, 5-year OS 7%. Risk factors identified in other studies have also included local tumor invasion (tongue or skin), high Ki-67, or EBV DNA titer >6.1 × 10⁷ copies/mL.

Radiotherapy is important in the management of patients with localized NK-/T-cell lymphoma with more favorable outcomes observed using high doses of radiotherapy (50–60 Gy) early in the frontline setting. Use of platinum-based concurrent chemotherapy as a radiosensitizer appears highly effective and may allow for the use of lower, less-toxic doses of radiation. Furthermore, because systemic relapse can occur with single-modality radiotherapy, other novel combinations are being tested. The outcome with CHOP has been disappointing, and it has been speculated that this may be due to overexpression of p-glycoprotein expression conferring multidrug resistance. Concurrent radiation (40 Gy) and cisplatin, followed by three cycles of VIPD (etoposide, ifosfamide, cisplatin), was evaluated in stage IE/IIE nasal NK-/T-cell

lymphoma. In this highly selected population, the CR rate was 83% and the 3-year PFS was 85%. Similarly, concurrent radiotherapy (50 Gy) and DeVic chemotherapy (dexamethasone, etoposide, ifosfamide, carboplatin) was evaluated with good results in a phase 1/2 trial in localized nasal NK-/T-cell lymphoma (CR 77%, 2-year PFS 67%). In the absence of a randomized trial, limited-stage patients may be treated with high-dose radiotherapy alone (>50 Gy) for stage 1 patients without risk factors or concurrent chemoradiotherapy (stage 1 or 2) using either of the noted regimens for localized NK-/T-cell lymphoma.

For advanced-stage disease, L-asparaginase has emerged as an active agent in NK-/T-cell lymphomas with an ORR of 87% (CR 50%) in relapsed or refractory patients. Antithrombin levels require close monitoring. A phase 2 study, evaluating L-asparaginase in combination with MTX and dexamethasone (AspaMetDex) in previously treated patients, demonstrates an ORR of 78% (CR 61%) and a median DOR of 12 months. A phase 2 study evaluating the SMILE regimen (steroid, methotrexate, ifosfamide, L-asparaginase, etoposide) in 38 patients with newly diagnosed stage IV or relapsed or refractory NK-/T-cell lymphoma demonstrated an ORR after two cycles of 79% (CR 45%); 19 patients subsequently underwent SCT. The 1-year OS rate was 55%, but grade 4 neutropenia occurred in 92% and the grade 3/4 infection rate was 61%. For patients with advanced-stage disease, who are sufficiently young and fit for intensive therapy, SMILE has emerged as preferred therapy. HDC/ASCT is also considered as consolidative therapy in advanced-stage patients. For patients with relapsed/refractory disease, PD-1 inhibition with pembrolizumab has demonstrated encouraging activity in small series and warrants further investigation.

Aggressive NK-cell leukemia

Aggressive NK-cell leukemia is a rare form of leukemia that almost always is associated with EBV infection and has a median survival of only 3 months. It is seen most often in Asians, and the median age of onset is 42 years. Typically, the bone marrow and peripheral blood are involved, in addition to the liver and spleen. Patients often have fever and constitutional symptoms and multiorgan failure with coagulopathy and hemophagocytic syndrome. It is unclear whether aggressive NK-cell leukemia represents the leukemic phase of extranodal NK-/T-cell lymphoma. There is no known curative therapy, and responses to chemotherapy are usually brief. Some encouraging results have been seen with L-asparaginase-based treatment in this disease as has been observed in patients with extranodal NK-/T-cell lymphoma, but this requires further study.

Uncommon aggressive PTCL subtypes

Subcutaneous panniculitis-like T-cell lymphoma. Subcutaneous panniculitis-like T-cell lymphoma (SCPTCL) is an uncommon PTCL subtype that preferentially infiltrates the subcutaneous tissue. It has been determined that tumors with the $\gamma\delta$ phenotype have a far inferior prognosis compared to those with the $\alpha\beta$ phenotype (5-year OS, 11% for $\gamma\delta$ vs 82% for $\alpha\beta$). In the WHO classification, SCPTCL is confined only to $\alpha\beta$ T cells, which usually have a CD4 $^-$ /CD8 $^+$ and CD5 $^-$ phenotype. Cases with a $\gamma\delta$ phenotype are combined in a new, rare PTCL entity termed *primary cutaneous $\gamma\delta$ T-cell lymphoma* (see section Primary cutaneous PTCL, rare aggressive subtypes) because of similar aggressive behavior. The optimal therapy for $\alpha\beta$ -type SCPTCL is unknown, with durable responses observed with both CHOP and immunosuppressive agents. Radiation therapy should be included for localized disease.

Hepatosplenic T-cell lymphoma. Hepatosplenic T-cell lymphoma is a rare PTCL subtype occurring usually in young men (median age 34 years) presenting with hepatosplenomegaly and bone-marrow involvement. Systemic “B” symptoms are common. Up to 20% of hepatosplenic T-cell lymphomas occur in the setting of immunosuppression, most commonly following solid-organ transplantation, and may occur a decade or longer after transplantation. It also has been observed in patients treated with azathioprine and the TNF α inhibitor, infliximab, which is used in Crohn’s disease. The splenic red pulp is diffusely involved, and the liver shows a sinusoidal pattern. Most tumor cells are CD3 $^+$, CD4 $^-$, and CD8 $^-$, and most are associated with isochromosome 7q. The majority of cases are of the $\gamma\delta$ TCR type; however, rare cases that are of the $\alpha\beta$ TCR type have been reported. The prognosis is extremely poor and long-term survival is rare. The optimal therapy is unknown; however, CHOP does not appear to cure this disease. High-dose cytarabine-based strategies, such as with IVAC (ifosfamide, etoposide, ara-c) have been reported to be more effective in case reports. Long-term survivors have been reported with allogeneic SCT, and referral for transplantation at diagnosis is suggested.

Enteropathy-associated T-cell lymphoma and monomorphic epitheliotrophic intestinal T-cell lymphoma. Recent findings have led to changes in the categorization of intestinal T-cell lymphomas. The two previously described variants of enteropathy-associated T-cell lymphoma (EATL) are now recognized as distinct; what was previously type II EATL is now designated as monomorphic epitheliotrophic intestinal T-cell lymphoma (MEITL). EATL is a rare, aggressive intestinal tumor, with a male predominance, that often occurs in

the setting of celiac disease and occurs typically in patients of northern European heritage. In contrast, MEITL shows no association with celiac disease and tends to occur in Asian and Hispanic populations. Both diseases commonly involve the jejunum or ileum with patients often presenting with abdominal pain; intestinal perforation can occur. The prognosis is extremely poor due to chemotherapy resistance and the difficulty of treatment delivery because of abdominal complications that can arise in the setting of malabsorption. In EATL, the neoplastic cells are typically polymorphous CD3 $^+$, CD7 $^+$, CD4 $^-$, CD8 $^{+/-}$, CD56 $^-$ $\alpha\beta$ T cells. In contrast, the neoplastic cells in MEITL are typically monomorphic CD3 $^+$, CD4 $^-$, CD8 $^+$, and CD56 $^+$ $\gamma\delta$ T cells.

The ITLP recently reported on 62 patients with intestinal T-cell lymphoma, which represented 5.4% of all lymphomas worldwide, occurring most commonly in Europe. EATL and MEITL represented 66% and 34% of the cases, respectively. The 5-year FFS was only 4% and OS was 20%, with the majority of patients treated with CHOP-type chemotherapy. Similar disappointing results are observed in other studies with CHOP-type therapy, which has prompted evaluation of HDC/ASCT (see Transplantation in PTCL below).

Primary cutaneous PTCL, rare aggressive subtypes

Primary cutaneous $\gamma\delta$ T-cell lymphoma. In the updated WHO classification, primary cutaneous $\gamma\delta$ T-cell lymphoma is now considered a distinct entity, which also includes cases previously known as SCPTCL with a $\gamma\delta$ phenotype, as described earlier. Clinically, the extremities are commonly affected, and the presentation can be variable, with patch or plaque disease or subcutaneous and deep dermal tumors that may exhibit necrosis and ulceration. The clonal T-cells have an activated $\gamma\delta$ cytotoxic phenotype and most are CD4 $^-$ /CD8 $^-$. Prognosis is poor in this disease, particularly with subcutaneous fat involvement, with a fulminant clinical course and chemoresistance.

Primary cutaneous aggressive epidermotropic CD8 $^+$ T-cell lymphoma. This provisional entity typically presents with generalized cutaneous lesions appearing as eruptive papules, nodules, and tumors with central ulceration and necrosis. Histologically, there is marked epidermotropism, and invasion into the dermis and adnexal structures is common. The tumor cells are CD3 $^+$, CD4 $^-$, CD8 $^+$, and cytotoxic marker-positive, and the clinical course is aggressive.

Transplantation in PTCL

Multiple retrospective studies have been published evaluating the impact of upfront transplantation in PTCL. Trial interpretation and comparisons are difficult for several

reasons, including the evaluation of heterogeneous patient populations, potential for selection bias, and the dearth of intention-to-treat (ITT) data. Because there are no reported prospective randomized phase 3 trials comparing HDC/ASCT with conventional-dose chemotherapy specifically for PTCL, it remains challenging to determine the relative impact of patient selection versus true differences in efficacy.

Several phase 2 prospective studies of upfront transplantation have been published and represent more homogeneous populations of treated patients. The Nordic Lymphoma Study Group completed the largest prospective phase 2 trial of upfront transplantation (NLGT-01) in 160 patients with PTCL, excluding ALK-positive ALCL. The planned treatment scheduled was CHOEP-14 for six cycles (CHOP-14 in patients >60 years old), followed by BEAM/BEAC and ASCT in responding patients. In total 160 patients represented the ITT population. Most patients had good functional status (71% with PS scores of 0 or 1), but 72% had an IPI score of >2. The CR rate pre-transplantation was 81% to transplantation, and the overall transplantation rate was 70% with a TRM of 4%. With median follow-up of 5-years, the 5-year PFS was 44% and 5-year OS was 51%. Patients with ALK-negative ALCL appeared to have a superior 5-year PFS (61%) compared with PTCL-NOS (38%), EATL (38%), or AITL (49%), but these results were not statistically significant. The 5-year OS for patients who underwent transplantation was 61% compared with 28% in those who did not. These results suggest that this approach may be appropriate in selected patients but it still represents level 2 evidence given the absence of data from a phase 3 trial.

For relapsed/refractory patients, HDC/ASCT represents the standard of care for eligible patients who have not undergone upfront transplant consolidation. In the original Parma study in which HDC/ASCT emerged as superior to second-line chemotherapy alone in relapsed aggressive NHL, immunophenotyping was not routinely performed. A subsequent report of prognostic factors did not identify a difference in outcome in B- versus T-cell lymphomas; however, the number of patients with PTCL was small. There has been no similar randomized study in PTCLs, but several retrospective studies report a salvage rate in this setting ranging from 18% to 60%. Given the overall body of evidence, ASCT frequently is offered to patients with PTCL with relapsed, chemosensitive disease.

Allogeneic SCT, with myeloablative or RIC, also has been reported to yield durable remission in many cases (3-year EFS, 23% to 64%). Evidence supporting a graft-vs-PTCL effect comes from studies with donor lymphocyte

infusions. The largest study published to date evaluated 77 previously treated patients with mainly myeloablative conditioning (74%). The 5-year PFS was 53%, but the TRM was 34% at 5 years. A phase 2 trial, evaluating RIC and allo-SCT in 17 patients, demonstrated a 3-year PFS of 64% with a TRM of 6%. Allogeneic transplantation is promising in the treatment of PTCL, but it is limited by the availability of stem-cell donors and by toxicity related to graft-versus-host disease.

Novel PTCL therapies

A number of agents are being explored in PTCL, three of which have FDA approval for use today in relapsed/refractory disease. Pralatrexate is a novel folate analogue that has enhanced uptake and cellular retention compared with MTX. Early studies suggested a sensitivity of TCLs over BCLs. The phase 2 PROPEL study evaluated pralatrexate (with vitamin B₁₂ and folate) in relapsed/refractory PTCLs and demonstrated an ORR of 29% (CR 11%), a median PFS of 3.5 months, and a median DOR of 10.5 months. The main toxicities were mucositis, thrombocytopenia, and neutropenia. These results led to FDA approval of pralatrexate in September 2009 for the treatment of relapsed/refractory PTCL. Of note, pralatrexate does not appear active in patients with AITL for whom other novel agents (HDAC inhibitors and brentuximab vedotin) are preferred.

As described previously, romidepsin is a HDAC inhibitor that has been evaluated in CTCLs and PTCLs. A phase 2B registration study was published evaluating romidepsin in 130 patients with relapsed or refractory PTCL. The ORR was 25% (CR 15%), median DOR was 17 months, and median PFS was 4 months, leading to FDA approval in 2011. Belinostat, another HDAC inhibitor, was FDA approved for relapsed/refractory PTCL in 2014 and demonstrates similar activity to romidepsin.

KEY POINTS

- BL should be treated with dose-intensive regimens which include CNS prophylaxis.
- Patients with congenital or acquired immunodeficiency have an increased risk of lymphoma and often respond poorly to therapy.
- PTCLs have an inferior outcome to DLBCL. The exceptions are ALK-positive ALCL and ALK-negative ALCL with *DUSP22-IRF4* rearrangements, which have a high cure rate with CHOP, CHOEP, or BV-CHP chemotherapy.

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Chronic lymphocytic leukemia/ small lymphocytic lymphoma

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Epidemiology

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is the most prevalent lymphoid malignancy in North America and Europe and is less common among people of African or Asian origin. It accounts for 25% to 30% of leukemia cases in the United States, with an estimated incidence of approximately 21,110 new diagnoses in 2017. The estimated prevalence of CLL in the US is 120,000 to 140,000 persons. The median age at diagnosis is 72 years, with an incidence rate in men twice that of women.

Biology

Cell of origin

CLL is an indolent malignancy of mature B cells. The cell of origin for CLL is not fully defined. CLL cells from patients with somatically hypermutated and unmutated immunoglobulin heavy-chain variable region (*IGHV*) (see “Pathophysiology” later in this chapter) have a similar gene-expression profile, suggesting a common cell of origin for CLL. Functional, immunophenotypic, and gene expression data suggest that CLL is most closely related to the CD5⁺ B-1 B-cell subpopulation. In human adults, B-1 cells constitutively produce polyreactive antimicrobial (natural) antibodies that are an important component of innate immunity.

Etiology

The cause of CLL remains unknown. There is considerable evidence to suggest a genetic predisposition to the disease. The risk of CLL in diverse populations is highly variable, with the highest risk in populations with northern European genetic heritage and a considerably lower incidence in populations of East Asian genetic heritage, irrespective of where they live. In addition, for the 5% to 10% of patients with familial CLL, their first-degree relatives have a significantly increased risk (~8.5-fold) of developing CLL or another B-cell malignancy. However, the clinical course of familial CLL in individuals with the disease is not determined by familial status, suggesting that the familial component pertains only to the risk of acquiring the disease. Genomewide genetic studies in familial and sporadic CLL cohorts have implicated over 40 germ line genetic polymor-



The online version of this chapter contains an educational multimedia component on chronic lymphocytic leukemia.

Conflict-of-interest disclosure:

Dr. Siddiqi: Speaker for ibrutinib (Pharmacyclics/Janssen) and brentuximab vedotin (Seattle Genetics); consultant for Juno Therapeutics, AstraZeneca, Pharmacyclics and BeiGene. Dr. Coutre: Consultant for Pharmacyclics, Janssen, Gilead, AbbVie, and Novartis.

Off-label drug use: CAR-T cells.

phisms, and it is therefore unlikely that CLL predisposition is related to a single genetic defect. Extensive studies have established that there are limited environmental risk factors for CLL.

Pre-CLL conditions

Aging is associated with major changes in both innate and adaptive immunity, including decreased antibody repertoire and increased frequency of oligoclonal B-cell populations. When people 60 years and older with normal complete blood counts are screened with high-sensitivity flow cytometry, >5% have small circulating monoclonal B-cell populations that are of unknown clinical importance. This condition is termed *monoclonal B-cell lymphocytosis* (MBL). By definition, MBL is not associated with organomegaly, lymphadenopathy, or abnormal blood counts. The high prevalence of this condition suggests that the development of CLL is a stepwise process affecting only a small percentage of patients with a preexisting monoclonal population of B cells. The presence of <50/ μ L clonal B lymphocytes is termed low-count MBL while the presence of >2,000/ μ L (but <5,000/ μ L) clonal B lymphocytes is termed high-count MBL. High-count MBL is associated with a 15% risk of developing CLL over a median of 6.7 years and is associated with risks of bacterial infections and secondary malignancies similar to those of CLL.

Pathophysiology

CLL is a disease typically characterized by peripheral blood lymphocytosis. When lymph-node involvement occurs, it is characterized by the progressive accumulation of monoclonal B cells that preferentially grow in the proliferation centers (pseudofollicles) of lymph nodes with an overall tumor-cell proliferation rate of 0.1% to 1% per day and prolonged overall cell survival (~3–6 months) because of defective apoptosis. An important driver of CLL survival and growth is B-cell receptor (BCR) signaling, and multiple mechanisms of sustained activation of the BCR in CLL have been described. Antigen-binding specificity of BCR is determined by the composition of the variable regions of the immunoglobulin molecule. Some CLL clones share BCRs with similar amino acid sequences (stereotyped BCRs) and this can be seen in about 30% CLL cases, primarily those with unmutated *IGHV*. These stereotyped BCRs have highly homologous heavy chain complementarity-determining region (CDR) 3s, often coded by identical *IGHV*, *IGHD*, and *IGHJ* segments. Many stereotyped BCRs also use the same *IGKV* or *IGLV*, such that the kappa CDR3s and lambda CDR3s are also very similar in protein structure. Studies have shown that CLL idiotype antibodies frequently react to autoantigens including antigenic targets

on apoptotic cells, tend to be polyreactive, and, in some cases, can even be activated by self-epitopes. These findings provide important insights into the biology of CLL and have also identified the BCR and its signaling pathway as therapeutic targets (see video in online edition).

Antigen-responsive B lymphocytes in the germinal center can be induced to undergo antigen-driven somatic hypermutation of the immunoglobulin genes, which alters epitope affinity for antigen. Somatic hypermutation of the variable region of *IGHV* is defined as ≥2% sequence difference from germ line and occurs in >50% of patients with CLL. CLL patients with these “mutated” *IGHVs* generally have a less aggressive disease course and better overall survival. In contrast, patients with CLL cells that have not undergone somatic hypermutation of *IGHV* (so-called unmutated CLL) generally have a more aggressive disease and poorer outcome, although this difference in prognosis may no longer exist with the new, oral-targeted drugs. Patients with mutated *IGHV* tend to have CLL cells that are anergic while patients with unmutated CLL have cells that are responsive to BCR cross-linking in vitro. However, the relationship between *IGHV* mutation status, BCR activation, and CLL disease biology is not yet fully understood.

CLL cells have apoptotic defects that contribute to increased survival in the stromal microenvironment of the lymphoid tissues and bone marrow. Important components of apoptosis resistance include upregulation of the anti-apoptotic molecules *BCL2* and *MCL1*. The molecular mechanisms of these defects are not fully understood. However, 13q14 deletion, the most common defect detected in CLL by interphase fluorescent in-situ hybridization (FISH), results in the deletion of genes coding for the inhibitory microRNAs (*mIR*) *mIR15* and *mIR16* that downregulate expression of the *BCL2* gene. The mechanism by which *BCL2* expression is upregulated in CLL patients without 13q14 deletion may be related to microRNAs.

Defects in the DNA damage-repair pathway in CLL cells are associated with more aggressive disease, cause resistance to DNA damaging chemotherapies, and increase the risk of disease transformation. These defects are an important but rare event in CLL patients at diagnosis (<10%). Defects increase in frequency with disease progression and occur in approximately 50% of patients refractory to therapies containing DNA-damaging chemotherapy. *TP53* defects disrupting p53 protein function occur either because of loss of one allele of *TP53* by 17p13 deletion and a dysfunctional mutation in the remaining *TP53* allele, biallelic dysfunctional mutations, or a single dominant negative mutation. Disruption of ATM function

can also result in a defective DNA damage-repair pathway in CLL cells. One allele of *ATM* is lost in the 11q22.3 deletion, and complete loss of function of *ATM* in these cells can occur because of disruptive mutations in the remaining allele. Loss of *ATM* function can also occur because of biallelic disruptive *ATM* mutations.

The pathophysiological effects of CLL cells are complex and not fully understood. Accumulation of CLL cells in the lymph nodes, spleen, and liver cause enlargement and disruption of function of these organs. Bone-marrow infiltration and the effects of CLL cells on myelopoiesis and the bone-marrow microenvironment can decrease hematopoiesis, resulting in cytopenias. CLL cells have an early detrimental effect on normal immune function. This results in impaired immunological response to infection, defective immunological self-recognition, and possibly defective immune surveillance for other malignancies. The mechanism of the constitutional effects of progressive CLL, including weight loss, drenching night sweats, fevers, and fatigue, are not fully understood but could be the result of dysregulated cytokine production.

KEY POINTS

- CLL is the most prevalent lymphoid malignancy in North America. The incidence of CLL increases with age.
- Risk of CLL is higher in populations of Northern European heritage; CLL is relatively uncommon in Asia.
- CLL is a familial disease in <10% of patients. Familial CLL does not increase the risk of a more aggressive disease course.
- Monoclonal B-cell lymphocytosis (MBL) is an established pre-CLL condition.

Diagnosis and clinical evaluation

Presentation

CLL, including the SLL variant, is usually diagnosed on evaluation of an incidental finding of asymptomatic leukocytosis/lymphocytosis or lymphadenopathy/splenomegaly. Only ~20% of patients have symptomatic disease at diagnosis. CLL can present with symptomatic anemia, bleeding due to thrombocytopenia, symptomatic adenopathy or splenomegaly (abdominal distention or early satiety), or constitutional symptoms. Constitutional symptoms include profound fatigue, drenching night sweats, fevers, and involuntary weight loss.

In the contemporary era, when patients are diagnosed earlier than in historical series, physical examination is often

normal at diagnosis. Possible physical findings include firm, rubbery nontender lymphadenopathy, which is frequently symmetrical, and palpable liver or spleen enlargement.

Diagnosis

Peripheral blood lymphocyte morphology

CLL cells have an appearance similar to normal small lymphocytes. CLL cells have increased cell-membrane fragility and tend to break during the process of making a blood smear, giving rise to smudge cells which are characteristic but not pathognomonic for CLL (Figure 24-1). A subset of circulating CLL cells can also have prolymphocytic morphology. Higher percentages of prolymphocytes in the peripheral blood (>55%) of patients with immunophenotypically diagnosed CLL have previously been considered to be indicative of transformation to “secondary” B-cell prolymphocytic leukemia (PLL). However, this finding could indicate clonal evolution of CLL with a *MYC* translocation or other adverse event rather than transformation to a distinct second disease. The latter would be quite rare.

Peripheral blood flow cytometry

The diagnosis of CLL can be made by immunophenotypic characterization of peripheral-blood lymphocytes by flow cytometry. B-cell clonality is determined by demonstrating light-chain restriction in the B ($CD19^+$) lymphocytes. CLL cells characteristically have dim CD20 and dim light-chain expression and coexpress CD5 and CD23. CD79b is a component of the BCR and expression usually parallels that of the light chain. Low CD20 expression

Figure 24-1 A peripheral blood smear of a patient with CLL (Giemsa stain; magnification $\times 400$) shows small lymphocytes and numerous smudge cells.

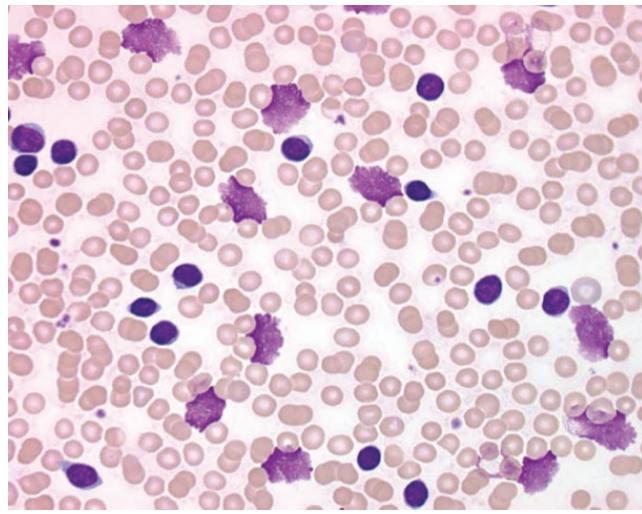


Table 24-1 Chronic B-cell lymphoproliferative disorders: immunophenotype

Disease	slg	CD20	CD5	CD23	CD10	CD103
Chronic lymphocytic leukemia	dim	dim	+	+	-	-
Lymphoplasmacytic lymphoma	+	+	-/+	-/+	-	-
Mantle cell lymphoma	+	+	+	-/dim	-	-
Nodal marginal zone lymphoma	+	+	-	-/+	-	-
Splenic marginal zone lymphoma	+	+	-/+	-/+	-	-/+
Follicular lymphoma	+	+	-	-/+	+/-	-
Hairy cell leukemia	+	+	-	-	-	+
B cell prolymphocytic leukemia	+	+	-/+	-	-	-

can be confirmed by a negative study with the low-affinity CD20-binding antibody FMC7. If the monoclonal B cells do not have the typical CLL immunophenotype (monoclonal B cells that are CD20 dim, light-chain dim, CD5⁺/CD23⁺), a wide differential diagnosis of other B-cell hematologic malignancies needs to be considered (Table 24-1). The leukemic phase of mantle-cell lymphoma is an important consideration and can be evaluated by FISH analysis for t(11;14).

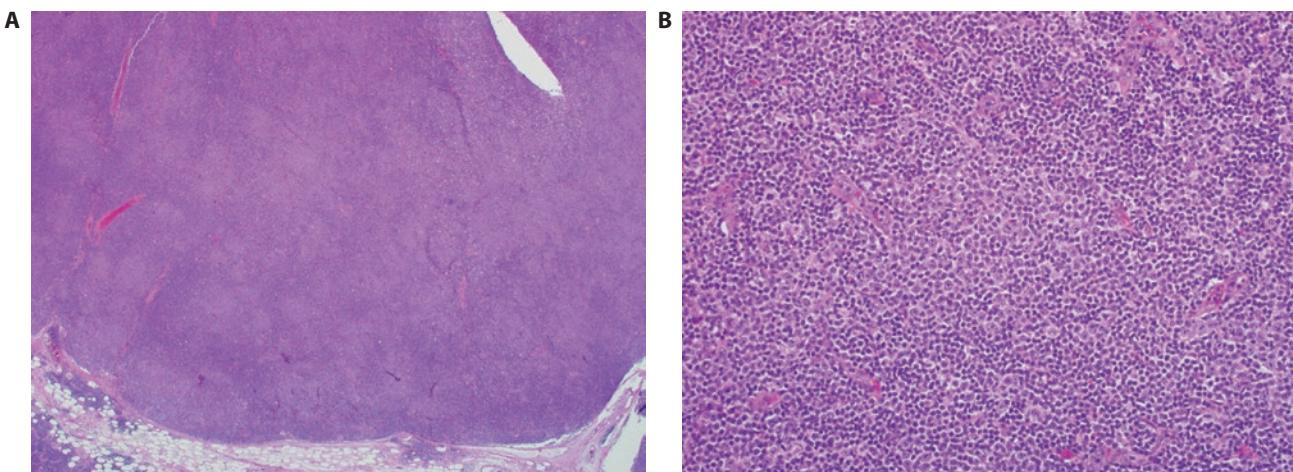
The International Workshop on Chronic Lymphocytic Leukemia (IWCLL) recently published updated guidelines for the diagnosis, indications for treatment, and response assessment of CLL. The guidelines require a peripheral-blood B-cell count of at least $5 \times 10^9/L$ to establish a diagnosis of CLL in a patient with a documented CLL immunophenotype monoclonal B-cell population. Patients with a similar clonal B-cell population, whose B-cell count is $<5 \times 10^9/L$ are considered to have the small lymphocytic lymphoma

(SLL) variant of the disease if they have lymphadenopathy or splenomegaly on physical examination or CT scanning or a mass with the same clonal B cells. Patients with a circulating monoclonal B-cell population with CLL immunophenotype who do not meet these criteria are considered to have clinical MBL. Assessing the B-cell counts in the peripheral blood requires quantitative-flow cytometric immunophenotyping.

Lymph-node biopsy

If a lymph-node biopsy shows SLL, there may or may not be detectable monoclonal B cells on peripheral blood-flow cytometry. Patients who require a lymph-node biopsy should have an excision or wide incisional biopsy because fine-needle-aspiration biopsy does not provide adequate tissue for architectural analysis of the lymphoid tissue. The pathognomonic characteristic of CLL/SLL is proliferation centers (pseudofollicles) (Figure 24-2).

Figure 24-2 Section of lymph node (hematoxylin and eosin stain) from a patient with CLL. (A) Low-magnification photomicrograph ($\times 20$) showing proliferation centers (pseudofollicles). (B) High-magnification photomicrograph ($\times 400$) of a proliferation center showing central large lymphocytes rimmed by small lymphocytes.



Bone-marrow study

Bone-marrow study is rarely required for the diagnosis of CLL. Lymphoid tissue is preferable to bone marrow for diagnostic purposes in patients with a nondiagnostic flow-cytometry immunophenotype. Bone-marrow studies can be helpful in assessing the etiology of cytopenias found in conjunction with the diagnosis of CLL but are otherwise of little value with the exception of assessment of minimal residual disease in the context of clinical trials.

Imaging

Baseline imaging studies, such as CT scans or PET scans, are not considered standard for most CLL patients at diagnosis.

Differential diagnosis

The differential diagnosis of leukemic-phase B-cell malignancies with small- to moderate-sized circulating lymphocytes with mature morphology (chronic B-cell lymphoproliferative disorders) includes CLL, mantle-cell lymphoma, splenic marginal-zone lymphoma, nodal marginal-zone lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, and B-cell prolymphocytic leukemia. These B-cell lymphoproliferative disorders can have distinct immunophenotypes (Table 24-1), but a definitive diagnosis can require additional testing (eg, FISH for t(11;14) for mantle-cell lymphoma; MyD88/CXCR4 mutation analysis for Waldenström's macroglobulinemia/lymphoplasmacytic lymphoma) or a diagnostic lymph-node biopsy.

Staging

Clinical staging using clinical evaluation and the complete blood count (Table 24-2) are useful for categorizing patients and identifying the small subpopulation of patients with advanced-stage disease that require therapy at the time of diagnosis. As noted above, CT or PET/CT scan results are not used for clinical staging.

KEY POINTS

- Flow cytometry is the gold standard for establishing the presence of clonal B cells with the CLL phenotype.
- The IWCLL criteria for the diagnosis of CLL require an absolute B-cell count of at least $5 \times 10^9/L$.
- A FISH probe for t(11;14) can help distinguish mantle-cell lymphoma from CLL.
- A bone-marrow biopsy is not required to diagnose CLL.

Risk stratification

Patients with CLL have a highly variable clinical course and outcome. Although the median time from diagnosis to first treatment is 5 to 7 years and median survival is >10 years, the wide ranges for these parameters limit the clinical utility of these data to plan patient management and provide accurate prognostic estimates. Because most CLL patients are now diagnosed with earlier stage disease, there is an important need for better prognostic markers. The most useful prognostic markers available utilize the biological characteristics of the patient's CLL cells.

Genetic analysis

CLL is characterized by recurrent genetic abnormalities that can be used to predict disease biology. The most commonly used analysis is FISH, which is a reliable, widely available, and relatively sensitive method of detecting specific chromosomal abnormalities in interphase cells. This methodology has been complemented by the clinical availability of conventional sequencing methods to detect abnormalities in individual genes of interest, and this methodology will likely be further expanded by the ability of next generation sequencing and array-based technologies, including CLL-specific mutation panels to provide rapid and affordable gene testing in the near future. This discussion focuses on methodologies that are currently clinically available.

Table 24-2 Clinical staging

Stage	Binet classification		Rai classification	
	Definition	Risk group	Stage	Definition
A	<3 lymphoid areas	Low	0	Lymphocytosis only
B	>3 lymphoid areas	Intermediate	I	Lymphadenopathy
			II	Hepato- or splenomegaly
C	Hemoglobin <10 g/dL or platelets <100 $\times 10^9/L$	High	III	Hemoglobin <11 g/dL
			IV	Platelets <100 $\times 10^9/L$

Karyotype analysis is a useful method of detecting chromosomal defects in dividing cells. Its ability to provide genetic information for CLL patients is limited by the low level of cell division in CLL cells, especially from patients with earlier-stage disease. CLL cells can be induced to divide in vitro using mitogens and Toll-like receptor (TLR) agonists, but these methods can cause artifacts and are not universally available; they are also not standardized. Although there has been recent interest in the use of complex karyotype to predict the disease course of patients with advanced stage CLL with treatment-refractory disease, the clinical role of these data still needs to be established.

FISH analysis provides an accessible method, using specific probes, for testing CLL cells for commonly recurring chromosome defects. The prognostic value of these data has been extensively studied and a hierarchical approach to ranking risk is clinically useful. Currently used genetic profiles use probes for 17p13 (*TP53* locus), 11q22.3 (*ATM* locus), trisomy 12, and 13q14 (*miR15A* and *miR16-1* loci). The hierarchical stratification for risk of disease progression is 17p13 deletion > 11q22.3 deletion > trisomy 12 > 13q14 deletion. Although this methodology is currently being modified by the addition of data from gene sequencing, the model continues to have clinical utility. Inclusion of a probe for 14q32 (*IGH* locus) can be useful for discrimination between CLL and mantle-cell lymphoma in leukemic phase. In addition, translocations involving *IGH* do occur in a small subpopulation of patients with CLL and are associated with an adverse prognosis.

Data from FISH analysis are limited by the probe set and the sensitivity of the assay. Most laboratories analyze all nucleated cells in the submitted sample. In early stage CLL, when the percentage of CLL cells in a blood specimen can be low, subclonal populations with a specific genetic defect can be present at a percentage below the detection threshold of FISH analysis (generally ~5%). In most patients, peripheral blood is the preferred sample for analysis. Bone-marrow aspirates usually contain a large number of nucleated red-blood-cell precursors that decrease assay sensitivity.

Gene sequencing has considerably improved the precision of analysis of genetic defects in the DNA damage-repair pathway in CLL. 17p13 deletion resulting in loss of one allele of *TP53* and 11q22.3 deletion resulting in loss of one allele of *ATM* usually affect only one chromosome, and the consequences of these deletions depend largely on the functional integrity of the remaining allele of *TP53* or *ATM*, respectively. Patients with 17p13 deletion (~5% of CLL at diagnosis, but more common in later-disease

stages) have an ~80% rate of dysfunctional mutations in the remaining *TP53* allele leading to loss of p53 function in those cells. In addition, disruption of p53 function in CLL can occur because of dysfunctional mutations in *TP53* in the absence of 17p13 deletion in ~5% of patients with CLL. These mutations can result in loss of p53 function because they are biallelic, associated with uniparental disomy, or because the gene product is dominant-negative and thus inhibits the activity of remaining normal p53. Patients with 11q22.3 deletion (~10% of CLL at diagnosis) have an ~30% rate of dysfunctional mutations in the remaining *ATM* allele, resulting in loss of ATM function and a poor prognosis. Patients with 11q22.3 deletion that retain a wild type *ATM* have a better prognosis than patients with loss of ATM function, but the former still have an inferior outcome compared to most patients with a monoallelic dysfunctional *ATM* mutation. This suggests that the 11q22.3 deletion results in loss of additional genes (eg, *BIRC3*) that can have adverse effects on prognosis.

Genomewide sequencing analysis of CLL has considerably improved our understanding of the molecular genetics of CLL. These studies identified several additional genes, including *NOTCH1* and *SF3B1* with recurrent mutations in CLL. Activating mutations of *NOTCH1* are detected in ~10% of patients with CLL at diagnosis, and these patients have more aggressive disease and a significantly increased risk of transformation to diffuse large B-cell lymphoma (DLBCL). Dysfunctional mutations in the gene coding for the splicing factor 3b subunit (*SF3B1*) of the spliceosome occur in ~10% of CLL patients at diagnosis and are associated with decreased duration of response to therapy and decreased OS.

Conventional sequencing for *TP53* mutations is clinically available and covers >90% of known defects in CLL. Use of this assay can increase the detection of *TP53* disruption in CLL at diagnosis. The *ATM* gene is very large and clinical sequencing is currently not available but could be in the near future. Conventional sequencing analysis is clinically available for analysis of *NOTCH1* and *SF3B1* mutations in patients with CLL. Current limitations to clinical use of gene sequencing in the routine evaluation of CLL patients at diagnosis are the cost of these assays, the still limited data on the utility of the more recently discovered prognostic mutations, and the absence of well-validated methods of integrating the data into a predictive model. In addition, the sensitivity of standard sequencing methods is limited to the detection of mutations that are present in >10% of the tumor-cell alleles, limiting the ability to detect small subclones of CLL cells which could have deleterious consequences in patients with early-stage disease.

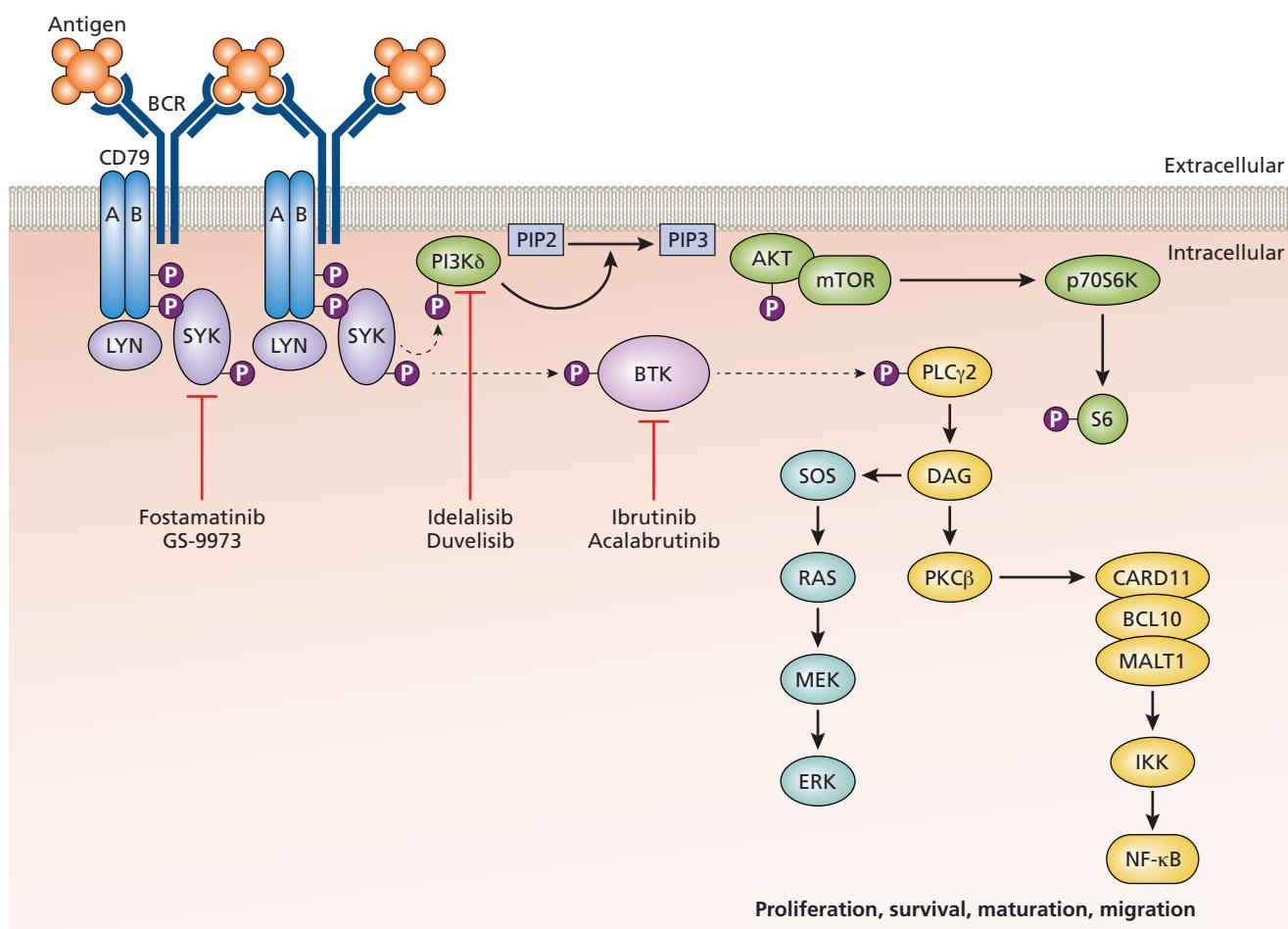
Clonal evolution and architecture

The CLL cell population frequently contains genetically defined subclones with the potential to expand and alter the course of the disease. Serial analysis with FISH showed that the apparent rate of detection of new subclones (clonal evolution) in an initially untreated CLL population was ~5% per year. Subsequent studies using considerably more sensitive (<1% allele frequency) next-generation sequencing (NGS) methods and array comparative genomic hybridization studies have shown a high rate of small subclones of cells with adverse genetic defects in previously untreated CLL patients. These data suggest that progression of CLL can be associated with clonal evolution where the architecture evolution results in subclone emergence with unfavorable genetic features. The role of evaluation of clonal complexity of the CLL cell population in clinical management is currently being investigated.

BCR analysis and stereotype

The BCR signaling essential for CLL cell survival and proliferation (Figure 24-3) can be modulated by *IGHV* somatic hypermutation and stereotype status. *IGHV* somatic hypermutation and VH family usage can be determined by standard sequencing in the clinical laboratory and does not change during the course of disease in CLL. Somatic hypermutation with gene sequence having <98% identity to germ line (mutated, ~55% of patients) is generally associated with less aggressive disease and longer survival compared to patients with ≥98% identity to germ line (unmutated, ~45% of patients). Patients with 97% to 98% germ-line identity should be considered to have borderline mutation because of the potential errors in sequencing and the arbitrary nature of the 2% cut-off that is used because of the difficulty in distinguishing

Figure 24-3 The B-cell receptor (BCR) comprises the idiotypic immunoglobulin and accessory signalling molecules Ig α (CD79A) and Ig β (CD79B). BCR activation induces signalling via a series of molecules to activate transcription factors (eg, NF- κ B) that promote cellular survival and proliferation. Signalling requires phosphorylation (P in purple circles) by protein kinases (pink symbols) and the lipid kinase PI3K δ (green symbol). Sites of pathway inhibition by targeted kinase inhibitors are shown. Redrawn from Wiestner A. *Hematology Am Soc Hematol Educ Program*. 2014;2014:125–134.



between mutations and unknown single-nucleotide polymorphisms (SNPs). Exceptions to this finding are patients with *IGHV* utilizing VH3-21 who have a poorer prognosis irrespective of mutation status.

Analysis of the immunoglobulin gene repertoire in CLL cells has contributed to a better understanding of the molecular pathogenesis of CLL. The recognition of a biased *IGHV* gene repertoire in CLL, distinct from normal B cells, and the discovery of specific antigen-binding sites among unrelated cases established the importance of antigen in the selection of CLL progenitor cells. Antigen-binding sites (VH complementarity determining region 3 [CDR3 regions] with high homology to previously described sites occur in ~20% to 30% of patients. These quasi-identical or stereotyped BCR can be classified into one of 19 major subsets, each of which has prognostic implications. The clinical implication of stereotype on management of CLL patients continues to be defined.

Prognostic markers of CLL cells

CLL cells can be analyzed for proteins that are differentially expressed in populations of patients with higher or lower risk of CLL progression.

ZAP70 is expressed by some normal and malignant B cells during differentiation and maturation and has a role in BCR signaling in CLL cells. ZAP70 assays were initially proposed as surrogate markers for *IGHV* mutation. However, subsequently studies showed a poor correlation (~70%) between these parameters, which is not clinically useful. However, higher levels of expression of ZAP70 are an independent marker of more aggressive CLL. Clinical use of this prognostic factor has been limited because accurate quantification of intracellular proteins by flow cytometry in clinical laboratories is technically difficult, and its use has largely fallen out of favor.

CD38 is a multifunctional surface molecule expressed by hematopoietic cells including B cells during maturation. CD38 is ligand of CD31 (PECAM1), also has enzymatic activity important for calcium metabolism, and can interact with the BCR/CD19 complex in B cells. CD38 expression by circulating CLL cells correlates with the rate of cellular turnover. Population studies show that higher levels of CD38 correlate with more aggressive disease and poorer outcome. However, the level of CD38 is not constant in patients with CLL, and there is difficulty determining the best cut-off for this continuous variable for risk stratification.

CD49d is the α_4 -integrin subunit that can associate with CD29 to form the $\alpha_4\beta_1$ -integrin (VLA-4). VLA-4, which is expressed by B cells including CLL, binds VCAM-1 (expressed by endothelial cells and bone-marrow stromal cells) and the extracellular matrix molecule fibronectin.

VLA-4 has an important role in trafficking of hematopoietic cells through the endothelium required to home to the lymph nodes and bone marrow. In CLL patients, increased CD49d expression is associated with a shorter time to first treatment and a poorer OS. Expression levels of CD49d are reported to be stable over time in individual patients. Although CD49d is the strongest flow-based predictor of overall survival, the availability and reporting of CD49d in clinical practice is variable.

β_2 -Microglobulin (B2M) is a polypeptide associated with HLA I on the cell membrane. Serum levels can be increased in several hematological malignancies including CLL. Increased serum-B2M levels that exceed 2 \times the upper limit of normal are associated with increased CLL tumor burden and shorter treatment-free and overall survival with chemoimmunotherapy. B2M is metabolized in the kidneys, and levels are increased in patients with renal impairment.

Lymphocyte doubling-time (LDT) is an estimate of time required for a patient's absolute lymphocyte count (ALC) to double. A clinically useful value requires a baseline ALC $>15 \times 10^9/L$ and 2 weekly counts over a period of at least 2 months. The LDT should then be calculated using linear regression. The initial studies in small patient cohorts reported in the 1980s concluded that a LDT of <12 months was associated with poorer prognosis. However, ALC is a labile parameter that is poorly predictive of the total tumor burden in CLL (<10% of CLL cells are in the circulation at any one time), and LDT should not be used as the sole parameter to predict a patient's prognosis or initiate treatment.

Prognosis at diagnosis

Developing an accurate and accessible prognostic evaluation system in newly diagnosed early-intermediate stage CLL patients has been challenging because of our rapidly changing understanding of the biology of the disease, the large number of potential prognostic factors, limitations of some of the published studies, and the indolent nature of the disease, which frequently makes the results of clinical studies of novel prognostic factors redundant before they are completed. In addition, factors, such as *TP53* disruption, which occurs in <10% of patients at diagnosis, are detected at low frequency with currently used clinical assays but are subsequently responsible for a disproportionate number of patients with more aggressive disease and poor outcome.

A new prognostic model combining genetic, biochemical and clinical parameters, called the CLL-International Prognostic Index (IPI), has recently been developed (Table 24-3). Following analysis of 27 baseline prognostic factors, the CLL-IPI Working Group determined that there are 5

Table 24-3 CLL-International Prognostic Index

Variable	Adverse factor	Score
Age	>65 years	1
Clinical stage	Binet B/C or Rai I-IV	1
17p13 deletion and/or TP53 mutation	Deleted and/or mutated	4
IGHV mutation status	Unmutated	2
B2M level (mg/L)	>3.5 mg/L	2

Prognostic scores range from 0–10 and identify 4 risk groups with significantly different rates of OS at 5 years ($p<0.001$ for all): low-risk patients (score 0–1), 93.2% (95% CI 90.5–96.0); intermediate risk (score 2–3), 79.3% (95% CI 75.5–83.2); high risk (score 4–6), 63.3% (95% CI 57.9–68.8); very high risk (score 7–10), 23.3% (95% CI 12.5–34.1).

independent prognostic markers for OS in CLL: TP53 (no abnormalities vs 17p13 deletion/TP53 mutations/both); *IGHV* mutational status (mutated vs unmutated); serum $\beta 2$ microglobulin (B2M) concentration ($\leq 3.5\text{mg/L}$ vs $> 3.5\text{mg/L}$); clinical stage (Binet A or Rai 0 vs Binet B-C or Rai I-IV); age (≤ 65 years vs > 65 years). Each marker was assigned a weighted risk score, and the combined score may allow for a more targeted management of patients with CLL.

KEY POINTS

- *TP53* disruption in CLL is associated with inferior prognosis.
- Patients with *IGHV* somatic hypermutation have superior survival compared to those without somatic hypermutation (unmutated).
- B2M levels may be elevated in the presence of renal impairment.

Management

Management of CLL is in rapid flux because of more accurate and earlier diagnosis, better risk stratification, recognition of complications and methods to prevent them, and the development of highly effective targeted therapies and immunotherapy.

At present there is no proven benefit to early treatment of patients with CLL. However, earlier diagnosis does allow for implementation of an active management plan to prevent complications of disease, early management of complications, and appropriate timing of treatment. Patients need to be well educated about their disease, the clinical manifestations of disease progression and complications, precautionary measures (see “Complications of CLL” later in this chapter), and measures to improve general fitness. The interval of patient follow-up can be determined by using

clinical monitoring and risk-factor analysis. The indications for initiation of treatment of progressive disease in both previously untreated patients and those with relapsed/refractory disease are based on the IWCLL guidelines (Table 24-4).

Goals of treatment should be considered for each patient. These goals may include improvement in disease-related signs and symptoms and quality of life, as well as prolongation of survival. As discussed below, current therapies are achieving deeper remissions, including minimal-residual-disease (MRD) negative responses. Such responses may significantly delay relapse, providing the rationale for MRD endpoints in ongoing clinical trials in an attempt to improve survival.

Monoclonal B-cell lymphocytosis

Patients with an incidental detection of a monoclonal B-cell population without symptoms, lymphocytosis, paraproteinemia, lymphadenopathy, or visceromegaly do not need further investigation or follow up. Patients with MBL with CLL immunophenotype and lymphocytosis, that do not meet the criteria for diagnosis of CLL, are considered to have clinical MBL with an annual ~1% to 2% risk of progression to CLL that would require treatment. These patients should be actively monitored in the same way as are patients with early-stage CLL. These patients have an increased incidence of infections and skin cancers and should be monitored as outlined below for all CLL patients.

Initial treatment of progressive CLL

Indications for treatment

Patients are considered to have active progressive disease requiring treatment if they have symptomatic disease, rapid disease progression, or bone-marrow failure as per the IWCLL guidelines (Table 24-4).

Evaluation of fitness

Choice of treatment for an individual patient depends on the biology of the patient’s disease and the patient’s physical fitness. Physical fitness should be determined using a minimum of standard evaluations of organ function (eg, estimated creatinine clearance) and performance status. Fitness-for-treatment should be assessed on an individual basis rather than by using chronological age alone. The role of more sophisticated methods of quantifying comorbidity and physical fitness, such as the cumulative illness rating scale (CIRS), are still investigational. Decreased fitness caused by potentially reversible CLL induced causes (eg, fatigue and symptomatic anemia) need to be carefully excluded from this evaluation. Fit patients should have an ECOG performance score (PS) of 0 or 1, no evidence of significant organ impairment, and no major comorbidity. Patients who are unfit, with PS ≥ 3 ,

Table 24-4 General indications for initiation of treatment in CLL (IWCLL 2018)

Indication	Description	Precautions
Bone marrow failure	Anemia (eg, Hb <10 g/dL) and/or thrombocytopenia (eg, $<100 \times 10^9/L$ and dropping)	Require bone marrow study to confirm bone marrow failure
Symptomatic disease	Unintentional weight loss >10% during the past 6 months Fatigue*: ECOG performance status ≥ 2 ; cannot work or perform usual activities Fevers $>38^\circ\text{C}$ for ≥ 2 weeks without evidence of infection Night sweats for >1 month without evidence of infection	Exclude other causative pathologies, eg, sleep disorder, depression, hypothyroidism, chronic infection/inflammation
Splenomegaly	Massive (>6 cm below the left costal margin) or symptomatic (abdominal distention, early satiety, pain) or progressive	
Lymphadenopathy	Massive (>10 cm in longest diameter) or symptomatic or progressive	Exclude infectious lymphadenitis and transformation to diffuse large B-cell lymphoma
Progressive lymphocytosis	Increase in absolute lymphocyte count (ALC) of $>50\%$ in 2 months or lymphocyte doubling time (LDT) of <6 months	Baseline ALC for calculation of LDT must be $>30 \times 10^9/L$. LDT needs to be determined by using multiple serial ALC counts (2 weekly ALC for >3 months) to perform linear regression analysis. All other potential causes of changes in ALC (eg, infection, recent use of corticosteroids) need to be excluded. ALC alone should not be used as an indication for treatment.
Autoimmune complications	Anemia or thrombocytopenia poorly responsive to corticosteroids	
Extranodal involvement	Symptomatic or functional, eg, skin, kidney, lung, spine	

*Use of fatigue as a sole indication for treatment of patients with CLL requires a careful evaluation and exclusion of all alternative etiologies.

major organ failure, or limiting comorbidity, should be considered for supportive and palliative care. Patients intermediate between fit and unfit (less fit) need to be considered for therapy options with lower toxicity.

CLL biological risk evaluation

As detailed above, *TP53* disruption by deletion and/or mutation (17p13 deletion/*TP53*^{mutation}) predicts poor response to chemoimmunotherapy and is an indication for alternative treatment approaches when treatment is indicated. CLL patients can have long intervals between diagnosis and treatment during which their CLL biology can be altered either by subclonal selection or by new mutations (clonal evolution). In patients without a previously demonstrated *TP53* disruption, a FISH panel, including a probe for 17p13 and *TP53* sequencing, should be performed within 6 months prior to initiation/change of treatment. The emergence of other mutations that can be detected by FISH, eg, del11q23, are also important and may influence choice of therapy. Therefore, we advocate obtaining a FISH panel (4–6 mutations) as well.

Evaluation of CLL disease burden

Evaluation of CLL disease burden before initiation of treatment is useful for planning therapy and evaluating response. Patients require a clinical evaluation of disease burden based on symptoms and physical examination with bidimensional measurement of the largest lymph node in the cervical, axillary, and inguinal/femoral regions on each side and the size of the liver and spleen (measured as centimeters below the costal margin at rest and at maximal inspiration in the midclavicular line). Imaging is not required to determine the size of the nonpalpable lymph nodes in the chest, abdomen, and pelvis except in clinical trials. CLL cells are usually not FDG avid, and PET scans should not be routinely used for CLL evaluation prior to initiation of therapy or for response assessment unless Richter's transformation is suspected.

Pretherapy precautions

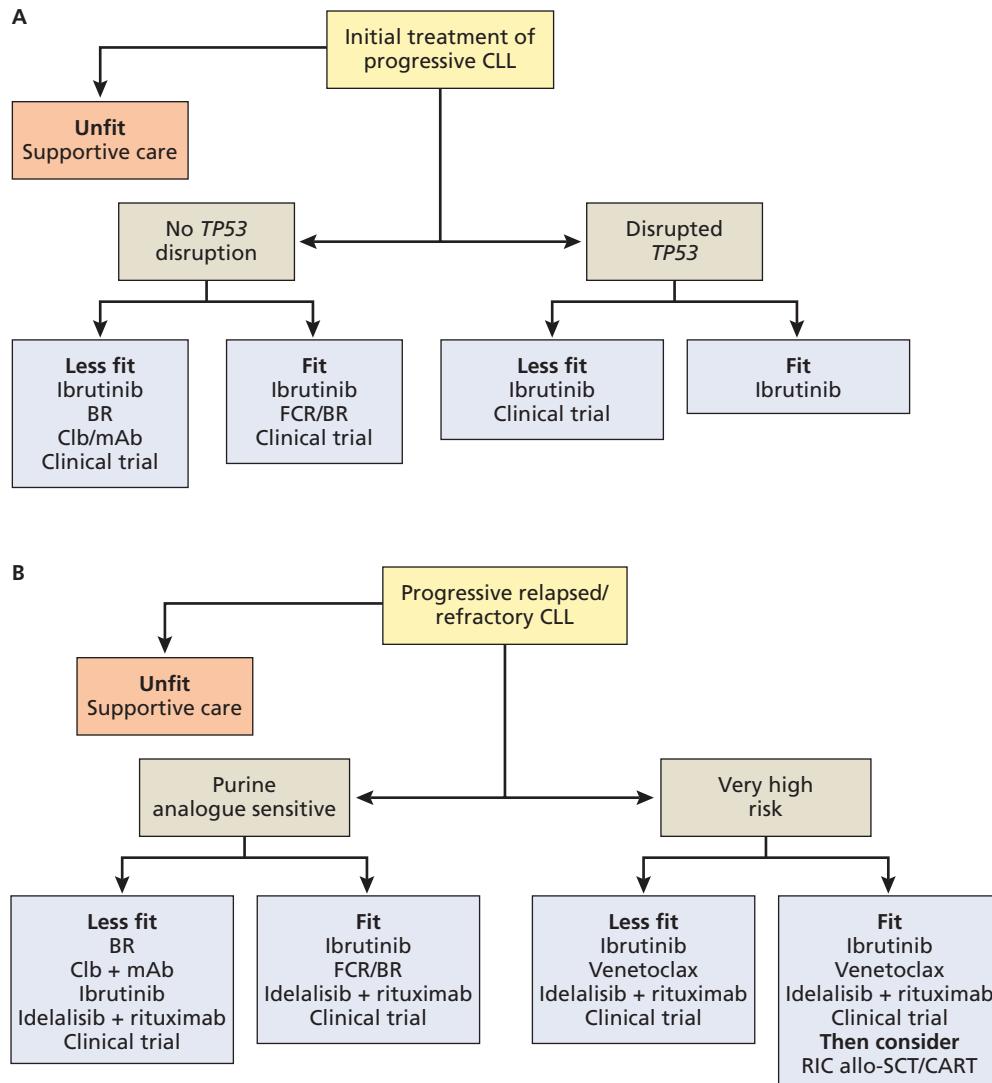
Use of monoclonal antibody therapy and myelosuppressive drugs increases the risks of reactivation of latent infections. CLL patients should be tested for evidence of infection

with hepatitis B and hepatitis C viruses before initiation of chemotherapy or use of monoclonal antibodies. Patients at high risk of reactivation (NCCN guidelines can be consulted to define this population) should receive antiviral therapy to minimize this risk. Patients could also benefit from anti-herpesvirus and anti-*Pneumocystis* prophylaxis with some regimens, although the value of these precautions is not proven. Effective therapy of CLL can cause

rapid cytotoxicity of CLL cells with toxic consequences, including tumor lysis syndrome. Prophylactic allopurinol and hydration together with appropriate monitoring are suggested particularly in patients with a high burden of disease at the start of therapy.

Patients requiring therapy for progressive CLL can be categorized according to CLL biology and physical fitness (Figure 24-4A). Genetic analysis identifying 17p13

Figure 24-4 Risk-stratified therapy for progressive CLL. (A) Initial selection of therapy for patients with progressive CLL should be based on patient fitness and the biology of the disease. Patients with predicted defective p53 function based on FISH analysis for 17p13 deletion or sequencing of *TP53* (disrupted *TP53*) should be treated with targeted therapy. Patients without disrupted *TP53* should be treated with chemoimmunotherapy (CIT) or ibrutinib. CIT regimens include fludarabine, cyclophosphamide and rituximab (FCR), bendamustine and rituximab (BR), pentostatin, cyclophosphamide and rituximab (PCR), and chlorambucil and anti-CD20 monoclonal antibodies (Clb + mAb). (B) Therapy for progressive relapsed/refractory disease is selected by using data on patient fitness, response to previous purine analogue containing CIT, and *TP53* status. Patients with purine analogue refractory CLL and those with disrupted *TP53* are considered very high risk.



deletion/*TP53*^{mutation} characterizes a subset of patients with very-high-risk CLL with poor- and short-duration responses to “conventional” chemoimmunotherapy (CIT)-containing regimens. These patients require alternative therapies. Patients in very poor physical condition are unlikely to benefit from CIT and should be considered for best supportive care. Alternatively, the newer novel targeted therapies can be considered.

Chemoimmunotherapy

CIT combining 1 or 2 chemotherapy agents (fludarabine, cyclophosphamide, bendamustine, chlorambucil) and anti-CD20 monoclonal antibodies (rituximab, ofatumumab, and obinutuzumab) is highly effective for most patients requiring initial therapy for progressive CLL. The combination of fludarabine, cyclophosphamide, and rituximab (FCR) utilizes the synergistic effect of combining cyclophosphamide with fludarabine, and the addition of rituximab to this chemotherapy combination has been shown to improve OS. The optimal choice of chemotherapy agents and monoclonal antibodies remains uncertain because of the rapid evolution of therapy for CLL; many of these therapies have not yet been studied in randomized controlled trials. The largest published experience with the longest follow up involves FCR, and, as such, this regimen is considered the standard of care among CIT regimens for patients with adequate performance status.

One large randomized trial (the German CLL Study Group’s CLL10 study), however, compared bendamustine and rituximab (BR) to FCR for initial treatment of fit patients. Patients treated with FCR had a significantly higher complete response (CR) rate and duration of response but also a higher risk of serious adverse events (eg, prolonged cytopenia and serious infections), especially in older patients. The study authors suggested that BR could be more tolerable and equally efficacious in fit patients older than 65 years and those with mutated *IGHV*. In younger, fit patients, FCR may yield long-term responses (>10 years) in a subgroup with no poor-risk features. In these patients, FCR may be an appropriate treatment.

Multiple alternative regimens combining alkylating agents and monoclonal antibodies can also be considered as initial therapy for less fit patients with CLL. Comparison of the results of the clinical trials using chlorambucil and anti-CD20 monoclonal antibodies is difficult because of the differences in the chlorambucil regimens. Although the addition of anti-CD20 monoclonal antibodies to chlorambucil regimens increases the rates of neutropenia and adds the additional risk of infusion reactions, the improvement in response suggests that chlorambucil should no longer be used as monotherapy. Chlorambucil combined with

rituximab resulted in an 84% overall response rate (ORR) with 10% CR rate in an elderly population (median age 70 years) with a median progression-free survival (PFS) of 24 months. The major adverse event was neutropenia (41% grade 3/4). A phase 2 trial of chlorambucil and rituximab in elderly patients (older than 65 years) had an 82% ORR with 19% CR and a median PFS of 34 months with use of maintenance rituximab. Addition of ofatumumab to chlorambucil monotherapy increased the ORR from 69% to 82% and the CR rate from 1% to 14% with significantly improved PFS. Chlorambucil monotherapy was compared to combinations with rituximab or obinutuzumab in the German-led CLL11 clinical trial for previously untreated patients with decreased fitness based on CIRS scores or decreased renal function. The results showed that combinations of chlorambucil with either obinutuzumab or rituximab significantly improved PFS and OS compared to chlorambucil monotherapy. There was significantly better PFS (29 vs 15 months) and time-to-next-treatment (43 vs 33 months) for obinutuzumab/chlorambucil vs rituximab/chlorambucil but no significant difference in OS. However, the optimal chlorambucil and anti-CD20 monoclonal antibody regimen has not yet been determined. Randomized clinical trials of BCR-signaling pathway-inhibitor-based regimens vs CIT regimens are discussed below.

Therapy for very-high-risk CLL

Patients with progressive CLL combined with 17p13 deletion/*TP53*^{mutation} have poor responses to drugs with a mechanism of action requiring an intact-DNA damage-response pathway. In patients receiving initial therapy with FCR in the German CLL8 trial, those with 17p13 deletion/*TP53*^{mutation} had a significantly inferior response compared to those without predicted *TP53* dysfunction: ORR (75% vs 98%), median PFS (15 vs 59 months), and median OS (42 months vs not-reached at median follow-up of 70 months). Similar poor responses were observed with other chemoimmunotherapies, including BR. The development of highly effective therapies with small-molecule targeted drugs that inhibit signaling pathways essential for CLL cell survival and growth has heralded a new era in the management of patients with CLL and are often considered the treatment of choice.

Targeted therapies

Ibrutinib targets Bruton tyrosine kinase (BTK) and is currently FDA-approved for initial treatment of patients with CLL as well as for those previously treated. Ibrutinib is discussed further below.

Idelalisib targets the phosphatidylinositol-4,5-bisphosphate-3-kinase catalytic subunit delta (PI3K δ), and is approved

in Europe in combination with rituximab for initial treatment of patients with CLL and 17p13 deletion/*TP53*^{mutation}. FDA approval in the US is only for treatment of relapsed/refractory CLL as described below.

Several other BTK and PI3K inhibitors are currently in development. An additional effective agent is the targeted small-molecule-inhibitor of BCL2 (venetoclax; ABT199) which was initially FDA-approved as monotherapy or with rituximab for patients with or without 17p13 deletion/*TP53*^{mutation} who have received at least one prior therapy. It is now FDA-approved for all relapsed/refractory CLL patients.

Other potential options are multidrug regimens (eg, ibrutinib with venetoclax and venetoclax with obinutuzumab) and the use of immune modulation by chimeric antigen-receptor T-cell (CAR-T) therapies as discussed below, although these approaches remain investigational.

Treatment of relapsed/refractory CLL

Patients with relapsed/refractory CLL can often be safely monitored until they meet the IWCLL criteria for progressive disease detailed in Table 24-3. Pretreatment evaluation of relapsed/refractory patients is similar to that required prior to initial treatment and includes evaluation of fitness, CLL biological risk, assessment of CLL disease burden, and screening for hepatitis B and C. An additional evaluation required for patients with relapsed disease is evaluation of the quality of the initial response to therapy. Patients who have previously responded to purine-analogue-containing CIT regimens with a response of at least 2 years' duration should be considered purine-analogue sensitive and can be considered for retreatment with similar CIT regimens. However, the duration of response to second treatment is usually shorter than to the initial therapy, and, given the impressive results with the targeted small molecule inhibitors, as well as the concern for treatment-related secondary malignancies after repeated exposure to chemotherapy, the newer agents are replacing CIT in this setting. Randomized clinical trials have shown that patients with a PFS of <2 years to purine-analogue-containing CIT and those with 17p13 deletion/*TP53*^{mutation} should be considered to be very-high-risk and should benefit from the targeted agents ibrutinib, venetoclax, or idelalisib and may benefit from other investigational agents in clinical trials (Figure 24-4B).

Patients with very-high-risk relapsed/refractory CLL previously had a very poor prognosis prior to the development of targeted small-molecule-inhibitor therapy. These drugs interrupt pathways required for CLL cell survival and proliferation, utilizing mechanisms that are independent of the DNA damage-response pathway and are thus

effective in patients with 17p13 deletion/*TP53*^{mutation} and those resistant to purine analogues.

Inhibitors of the BCR pathway (Figure 24-3) are a unique class of drugs that are highly effective in CLL and have changed practice. Their introduction has required an ongoing re-evaluation of the role of prognostic factors in predicting response to treatment, revision of response criteria, changes in duration of therapy, and the need to recognize and manage different adverse events. Targeted inhibitors of BCL2 represent yet another treatment option that is also changing practice and is also effective in patients who have previously received BCR pathway inhibitors.

BTK inhibition: ibrutinib

BTK is an important component of the BCR signaling pathway (Figure 24-3) expressed in hematopoietic tissue except T cells and plasma cells. Ibrutinib is an orally administered molecule that binds covalently to a cysteine residue near the enzymatic site of BTK resulting in irreversible inhibition. Although ibrutinib has a short half-life, BTK binding is irreversible, and cellular BTK enzymatic activity can be restored only by synthesis of new BTK protein which extends the therapeutic effect, allowing once-daily administration. Ibrutinib is FDA-approved as both initial therapy and for patients with previously treated CLL. Onset of action is rapid, with resolution of symptoms and decreases in lymphadenopathy and visceromegaly within days of starting therapy, followed by a slower but progressive recovery from cytopenia. Therapy is frequently (>70%) associated with exacerbation of lymphocytosis due to redistribution that does not affect response to therapy, usually peaks after one month of therapy, and subsequently slowly declines. The median time to resolution of lymphocytosis on ibrutinib therapy is 19 weeks, but prolonged lymphocytosis up to 124 weeks has been seen in patients with ongoing treatment responses. Treatment-related lymphocytosis is a class effect associated with use of drugs that inhibit BCR pathway signaling and does not require specific management.

Response

Ibrutinib monotherapy is very effective as initial therapy for older, fit patients who require therapy. Experience as initial therapy in young patients, or in those who are less fit is more limited. Large, randomized trials comparing ibrutinib to CIT (FCR and BR) in young patients are ongoing. Ibrutinib has also been highly effective in patients with relapsed/refractory CLL with limited alternative options. However, direct comparisons of response rates with other previously used therapies have been difficult because of the slow but ongoing response to ibrutinib therapy

in many patients and the difficulty in response evaluation in patients with increasing or persistent lymphocytosis. These difficulties have been partially overcome by the revision of the standard IWCLL response criteria to include the new category of “partial remission with lymphocytosis” (PR-L). Ibrutinib monotherapy of patients with relapsed/refractory CLL, including many with very-high-risk disease (17p13 deletion/ TP53^{mutated} and purine analogue refractory), has achieved high response rates (ORR ~90%). Although most of these responses were PR or PR-L (~80%) with low CR rates (<10%), CR rates continued to increase with ongoing therapy with a median time to CR of 21 months in one study. The duration of response is considerably better than those reported for previously used therapies with an estimated 30-month PFS of 69% and OS of 79%. Both PFS and OS appear to be inferior in patients with 17p13 deletion (and possibly also in patients with 11q22.3 deletion). In contrast, initial studies have not shown major differences in response rates and duration of response based on other prognostic factors used to assess disease risk in CLL including *IGHV* mutation status, expression of CD38 and ZAP70, and other FISH-determined genetic abnormalities. Ibrutinib monotherapy thus provides a highly effective but noncurative option for patients either as initial therapy or for relapsed refractory CLL. The challenge is to learn how to use this novel agent most effectively, improve its efficacy, and determine if there is a subgroup of responding patients who will not have rapid disease progression if therapy is discontinued.

Toxicity

Ibrutinib is generally well tolerated. Diarrhea and skin rashes are relatively common, often transient, and can resolve with no specific management. Less common but more serious drug-specific complications include bleeding, atrial fibrillation, arthritis and arthralgia, fatigue, cytopenias, and infections. BTK signaling is important for platelet activation, and ibrutinib decreases platelet adhesion to von Willebrand factor, increasing the risk of bleeding. Ibrutinib therapy is frequently complicated by minor bruising. Severe hemorrhages are less common, and patients on anti-coagulant and antiplatelet therapy are at the highest risk of these complications. Ibrutinib therapy should be stopped for 3–7 days before and after surgical procedures because of the risk of bleeding. Atrial fibrillation is an important complication of ibrutinib therapy (~10%) that could be the result of the inhibition of BTK and related kinases (eg, TEK). Patients with relapsed/refractory disease CLL have high rates of infections, but ibrutinib does not appear to contribute to this risk. Ibrutinib is metabolized by cyto-

chrome P450 enzyme 3A (CYP3A), and potential drug interactions need to be considered in its use. Concomitant use of moderate or strong CYP3A inhibitors requires ibrutinib dose-reductions.

Resistance

Patients with relapsed/refractory CLL can acquire resistance to the drug after an initial response to treatment. Transformation to diffuse large B-cell lymphoma or Hodgkin lymphoma (Richter's transformation) is observed in <5% of treated individuals and tends to occur within the first 6 months of therapy. Acquired resistance to ibrutinib therapy is largely due to mutations that prevent ibrutinib from inhibiting BCR signaling. Two such mutations have been described, a cysteine to serine change at amino acid 481 in BTK that prevents ibrutinib binding to the active enzymatic site and a gain of function mutation in the gene coding for PLC γ 2 that results in autonomous BCR signaling. Although the total number of mutations is low, these mutations were found in 85% of heavily pretreated patients who experienced disease progression while receiving ibrutinib therapy. Disease progression on ibrutinib therapy is most frequent in patients with 17p13 deletion/TP53^{mutation} as well as with complex karyotypic abnormalities (>1 aberration). These BCR pathway mutations have not yet been detected in patients with CLL prior to initiation of treatment with ibrutinib, suggesting that mutations occur either because of new mutations or by selection of pre-existing subclones of ibrutinib-resistant cells too small to detect by current assays.

If ibrutinib is stopped due to progressive disease, rapid disease progression can occur. Therefore, for patients who have an indication for immediate therapy, it is appropriate to continue ibrutinib until an alternative therapy is started.

Combination therapy

Ibrutinib combination therapy is being tested in clinical trials. Combination with anti-CD20 monoclonal antibodies is potentially attractive because monoclonal antibodies are most effective at killing circulating CLL cells. However, enthusiasm for these combinations is tempered by data suggesting that ibrutinib could decrease cell-mediated monoclonal antibody-dependent cytotoxicity. A recent trial involving previously treated patients demonstrated no improvement in PFS with the addition of rituximab to ibrutinib. Combinations of ibrutinib with anti-CD20 monoclonal antibodies, venetoclax, and CIT are currently being tested in clinical trials of both relapsed and previously untreated patients. A full listing of ongoing trials can be found at clinicaltrials.gov.

PI3K δ Inhibition: idelalisib

The PI3K p110 δ (PI3K δ) enzyme is expressed primarily in hematopoietic tissue and is especially important for B-cell maturation and survival. In CLL, PI3K activity is constitutively activated suggesting that it could be a good target for therapy. Specific inhibitors of PI3K δ could thus provide targeted therapy for CLL. Idelalisib is an orally bioavailable selective inhibitor of PI3K δ which also inhibits CXCR4 and CXCR5 signaling. Inhibition of PI3K δ prevents phosphorylation of the serine/threonine kinases AKT and mTOR resulting in decreased BCR-pathway signaling (Figure 24-3).

Response

Idelalisib is FDA-approved for CLL therapy in combination with rituximab for relapsed CLL patients with comorbidities and for treatment of patients with relapsed SLL or follicular lymphoma who have received at least two prior therapies. Idelalisib monotherapy for patients with relapsed/refractory CLL (70% refractory to previous treatment and 24% with 17p13 deletion/TP53^{mutation}) resulted in an ORR of 72% with 39% PR and 33% PR-L and a median PFS of 15.8 months. A randomized controlled study tested idelalisib and rituximab vs rituximab alone in CLL patients with relapsed/refractory disease and comorbidities that precluded the use of CIT. Addition of idelalisib to rituximab significantly improved ORR, PFS, and OS. A subsequent update of this study showed that response rates and PFS in the patients receiving idelalisib and rituximab were not affected by 17p13 deletion/TP53^{mutation},IGHV mutation status, or levels of ZAP70 expression. Excess hepatotoxicity and increased mortality due to infections resulted in the discontinuation of several randomized trials with idelalisib as initial therapy. As a result, there are no extensive data on the efficacy of idelalisib for previously untreated CLL patients. A phase II trial enrolled patients age 65 years or older, with previously untreated CLL, who received either idelalisib or idelalisib with rituximab. Toxicity, including significant colitis as well as infections, limited its long-term use.

Toxicity

Idelalisib inhibition of BCR signaling causes the class effect of lymphocytosis that is unlikely to have any clinical significance. Idelalisib does have important potential toxicity that requires careful monitoring of patients. Gastrointestinal complications include diarrhea, that can be severe and nonresponsive to motility-inhibiting drugs, severe colitis, and intestinal perforation. Hepatic toxicity includes frequent transaminitis that usually resolves on drug cessation and, less commonly, more severe hepatitis that requires intervention. Severe hepatotoxicity was noted primarily in young, previously untreated patients. Pneumonitis requiring drug cessation, and treatment with corticosteroids has been reported. Additional toxicities include pyrexia, fatigue, nausea, rash, neutropenia, hypertriglyceridemia, and hyperglycemia. Idelalisib has been shown to increase the risk of infection, including CMV infection or viremia and *Pneumocystis jirovecii* pneumonia, infections that are typically seen in immunocompromised patients. Careful monitoring is required. Idelalisib induces CYP3A, and thus the risk of adverse drug interactions must be considered.

BCL2 inhibition: venetoclax

Venetoclax is an orally active, targeted small-molecule inhibitor of the anti-apoptotic molecule BCL2 that is expressed at high levels in CLL cells. Venetoclax monotherapy, in combination with rituximab for treatment of patients with relapsed/refractory CLL, is reported to achieve ORR in excess of 80% with CR rates of ~30%, with some patients achieving MRD-negative status. Responses were similar in patients with 17p13 deletion. The major toxicity was severe tumor lysis, but the risk of this complication has been decreased by a revised administration regimen. Venetoclax was initially FDA-approved for previously treated patients with 17p13 deletion CLL and has now received broader approval in the relapsed setting based on the phase 3 Murano trial comparing venetoclax plus rituximab to bendamustine plus rituximab. The venetoclax plus rituximab arm showed significantly better progression-free survival and overall response rates. It has also demonstrated a benefit in patients previously treated with either ibrutinib or idelalisib, who have either become resistant to, or intolerant of, these therapies. It is being studied as initial therapy in combination with ibrutinib and/or anti-CD20 monoclonal antibodies.

Immunotherapy

Restoring immune surveillance and immune-based cytotoxicity capable of preventing recurrence of CLL in patients who have minimal residual disease after effective therapy can result in long-term disease control and possibly even cure. The first effective modality was reduced-intensity conditioning (RIC) allogeneic stem-cell transplantation (allo-SCT) in selected patients; chimeric antigen receptor T-cell (CAR-T) therapy is now being evaluated in clinical trials.

RIC allo-SCT is effective therapy in relapsed/refractory CLL for patients with very-high-risk disease. However, therapy is complicated by chronic graft-versus-host disease with

treatment-related mortality of ~20%. Optimum results are achieved in younger and fitter patients with minimal residual CLL who have not had extensive prior therapy. The availability of highly effective targeted small-molecule therapies and alternative immunotherapies has reduced the enthusiasm for RIC allo-SCT in CLL; indications for use of this therapy are currently unclear. Patients with very-high-risk CLL, who are candidates for immune therapy, should be referred for evaluation at a center specializing in the treatment of CLL early in the course of their disease.

CART therapy

Ex-vivo introduction of chimeric genes into autologous T cells using lentivirus vectors can induce “autologous” anti-CLL immunity. The chimeric gene construct code for antibody variable regions (eg, B-cell specific anti-CD19 or more CLL specific anti-ROR1) together with immunostimulatory molecules (eg, CD3z, CD28, CD137). CAR-T cell therapies are being evaluated in ongoing clinical trials after promising initial results were observed in CLL.

KEY POINTS

- FCR chemotherapy has a higher CR rate and duration of response than other CIT regimens but may have an increased risk of serious adverse events in less fit patients and those older than 65 years.
- FCR as initial therapy for patients with mutated IGHV and non-17p13 deletion can result in very prolonged survival.
- Ibrutinib targets BTK and is approved by the FDA for both initial treatment and for relapsed/refractory CLL.
- Ibrutinib is metabolized via CYP3A, and concomitant use with CYP3A inhibitors requires dose reductions.
- Idelalisib with rituximab is approved for treatment of relapsed/refractory CLL in less fit patients, but the substantial risks of colitis and serious infections limit its use.
- Idelalisib should not be used as initial therapy due to severe hepatotoxicity, particularly in young patients.
- Venetoclax is approved for relapsed 17p13 deletion CLL but can cause tumor lysis syndrome in patients with high disease burden which is why a gradual ramp-up of dosing is employed.

Complications of CLL

The course of CLL is complicated by defective innate and acquired immune function that develops early in the clinical course of the disease. This immune dysfunction generally becomes more severe with disease progression and is

exacerbated by conventional therapies. Immunodeficiency increases the risk of infection and autoimmune disease, and defective immune surveillance could contribute to the increase risk of second malignancy. CLL patients are also at increased risk of clonal evolution to aggressive lymphoma (Richter's transformation).

Infections

Serious infections result in considerable morbidity and are a major cause of death in CLL patients. Defective responses to antigens by nonmalignant B cells results in quantitative and qualitative defects in antibody production. Although absolute T-cell counts are usually increased in patients with CLL, CD4/CD8 ratios are reversed with decreased T-cell receptor repertoire and markedly impaired T-cell function. Innate immunity is impaired by monocyte, dendritic, and NK cell dysfunction; decreased serum-complement levels; and bone marrow failure-associated neutropenia.

Clinical

Impaired humoral immunity markedly increases the risk of overwhelming bacterial infections by encapsulated organisms (eg, *Streptococcus pneumoniae* and *Staphylococcus aureus*) at all stages of CLL. Defective T-cell immunity increases the risk of herpesvirus reactivation. Reactivation of varicella-zoster virus results in shingles, which is frequently complicated by postherpetic neuralgia and can also lead to disseminated varicella-zoster. Herpes simplex virus reactivation can result in local lymphadenitis and systemic herpes simplex virus infections. Cytomegalovirus reactivation is more common in patients with advanced-stage disease and those treated with lymphotoxic therapies. CLL patients with advanced-stage disease and those undergoing immunosuppressive therapy or allogeneic hematopoietic stem-cell transplantation are at high risk of fungal and atypical bacterial infections. Idelalisib is associated with significant infectious complications, as discussed above. Ibrutinib has also been associated with early-onset fungal infection, especially in patients with other predisposing risk factors, including the use of corticosteroids.

Prevention

Preventative measures, education, and rapid and effective responses to infection can decrease the risk and consequences of serious infections. Patients need to be trained to recognize and to seek immediate medical evaluation for serious infections and especially fevers. Vaccination responses are usually suboptimal in patients with CLL. However, pneumococcal vaccine responses can be improved by addition of the conjugated 13-valent vaccine to the standard 23-valent

polysaccharide vaccine. Influenza vaccines are likely to be of most value in patients with early-stage CLL but should be administered to all patients and household members, if possible. Live virus vaccines (eg, yellow fever) are contraindicated.

Prophylactic antimicrobial therapy is not of proven value in CLL. Pneumocystis and herpesvirus prophylaxis is commonly used during and after therapies with lymphotoxic drugs (purine analogues and high-dose corticosteroids). Prophylactic antiviral therapy can be useful in decreasing the risk of varicella-zoster and herpes simplex virus reactivation in patients with recurrent infections. A recombinant varicella-zoster vaccine is now available (Shingrix), which is likely safe to use in CLL patients, in contrast to Zostavax, which carries a risk of viral infection because it is an attenuated virus vaccine.

The use of intravenous immunoglobulin (IVIG) in management of CLL is not well established. IVIG 0.4 mg/kg every 4 weeks has been shown to decrease the risk of infections but may not extend OS. IVIG can cause serious adverse events and is expensive. Its use should probably be limited to patients with recurrent major infections (at least 2 in 6 months) and should not be based on IgG levels alone. Subcutaneous formulations are also available for home use.

Effective management of infections in patients with CLL can be challenging. Infection evaluation should focus on encapsulated bacteria and atypical and opportunistic infections. Treatment should be based on the assumption that all CLL patients are immune-compromised. The NCCN Clinical Practice Guidelines for the Prevention and Treatment of Cancer-Related Infections provides comprehensive recommendations.

Autoimmune disease

Approximately 5% to 10% of CLL patients have autoimmune complications, most of which are hematological (eg, autoimmune hemolytic anemia or immune thrombocytopenia).

Hematological disease

Most (>90%) autoimmune cytopenia is caused by loss of self-tolerance attributed to disruption of T-cell function by CLL cells. This disruption causes pathological production of high-affinity polyclonal IgG antibodies directed against blood-cell antigens by nonmalignant B cells resulting in autoimmune hemolytic anemia (AIHA) or immune thrombocytopenia (ITP). In contrast, production of a self-reactive monoclonal antibody (usually IgM) by CLL cells is rare and occurs in <10% of patients with AIHA or ITP. Pure red-blood-cell aplasia (PRCA) can be mediated by

either autoantibodies or direct T-cell cytotoxicity. Autoimmune cytopenias occur throughout the course of CLL and cause 15% to 20% of noniatrogenic cytopenias in CLL patients. Patients with autoimmune cytopenia should not be classified as having advanced-stage disease unless they have concomitant bone-marrow failure demonstrated by bone-marrow biopsy.

AIHA

Clinical. AIHA is usually characterized by reticulocytosis in the absence of bleeding, elevated serum LDH and indirect bilirubin levels, and a positive direct antiglobulin test (DAT) that detects surface-bound anti-red blood cell IgG antibodies and the complement degradation product C3d. However, patients with AIHA- and CLL-related bone-marrow failure (complex AIHA) are often not able to generate a reticulocyte response to anemia. In addition, although DAT tests are positive in >90% of CLL patients with AIHA, ~15% to 20% of CLL patients have a positive DAT during the course of their disease, and only 35% of these patients develop AIHA.

Management. Patients with AIHA and adequate erythropoiesis (simple AIHA) can be treated with immunosuppression using corticosteroids. Patients with severe anemia or a slow response to corticosteroid therapy can benefit from addition of IVIG. AIHA relapses are common, and many patients require long-term immunosuppression or additional treatment, such as anti-CD20 monoclonal antibodies. Patients with both AIHA- and CLL-related bone-marrow failure require treatment for both conditions. Because purine analogues are myelosuppressive and can cause autoimmune cytopenia when used as monotherapy, these agents should probably be avoided. Therapy with ibrutinib has also been shown to be very effective in management CLL associated AIHA. Splenectomy is less effective here than in patients with idiopathic AIHA.

ITP

Clinical. CLL patients with progressive bone-marrow failure usually develop anemia first and thrombocytopenia subsequently. CLL patients presenting with thrombocytopenia without anemia should be evaluated for causes of platelet sequestration. A bone-marrow examination may be helpful in this scenario. In patients with insidious-onset thrombocytopenia and platelet counts >50 × 10⁹/L, hypersplenism should be considered. In contrast, acute onset (<2 weeks) or severe thrombocytopenia (platelet counts <30 × 10⁹/L) in CLL patients is more likely to be caused by ITP. Anti-platelet antibody assays have low specificity

and sensitivity and are not useful in making the diagnosis of ITP, which remains one of exclusion.

Management. Patients with no bleeding complications and platelet counts $>20 \times 10^9/L$ should be carefully observed and educated, but they do not need active treatment. Those needing treatment usually respond to immunosuppression with corticosteroids. Thrombopoietin agonists can be useful if patients have a slow or inadequate response to immunosuppression. Splenectomy is considered less effective in CLL patients compared to primary ITP. Patients with ITP and bone-marrow failure can be treated with regimens similar to those used to manage complex AIHA. Caution is advised with use of ibrutinib in the presence of severe thrombocytopenia because of the increased risk of bleeding.

PRCA

Clinical. Autoimmune PRCA presents with anemia, a very low absolute reticulocyte count, and no evidence of hemolysis or bleeding. A definitive diagnosis requires a bone-marrow study showing an erythroid-lineage maturation arrest. The differential diagnosis includes parvovirus and other virus infections. Because patients with CLL have inadequate humoral immune response to infections, detection of parvovirus and CMV viremia by PCR is more useful than viral serology.

Management. PRCA should be treated with immunosuppression using prednisone and cyclosporine. Clinical improvement is often slow because of the lag time to restoration of erythropoiesis. Long-term immunosuppression is frequently required to maintain adequate hemoglobin levels.

Autoimmune neutropenia

This is a rare and poorly understood condition that should be considered in patients with isolated neutropenia of uncertain etiology, especially if it is severe. A bone-marrow examination should be considered to help in the differential diagnosis. Large granular lymphocyte-associated neutropenia should also be considered.

Nonhematological disease

Patients with CLL have an increased risk of autoimmune-acquired angioedema, paraneoplastic pemphigus, and glomerulonephritis. A clinically important consequence of immune dysregulation in CLL patients is exaggerated cutaneous arthropod-bite reactions which can be complicated by cellulitis and transient painful adenopathy, often mistaken by patients for disease progression.

Second malignancies

Hematological malignancies

Lymphoid malignancies

DLBCL can occur at any time in the course of CLL (Richter's transformation, incidence ~0.5% per year) with the highest risk in patients with *NOTCH1* mutations and 17p13 deletion/*TP53*^{mutation}. In ~80% of patients with CLL, who develop a DLBCL, a CLL cell undergoes clonal transformation to a highly aggressive DLBCL with very poor prognosis. In contrast, ~20% of CLL patients developing DLBCL have clonally unrelated de novo DLBCL with a considerably more favorable prognosis. These two etiologies can be distinguished by VDJ rearrangement analysis of paired CLL- and DLBCL-cell samples. Diagnosing de novo DLBCL is challenging. Testing for clonality may not be readily available to the practitioner. Patients with CLL are also at increased risk of developing Hodgkin lymphoma and other B-cell malignancies.

Management. CLL patients diagnosed with de novo DLBCL require standard evaluation and management. There is no standard of care for clonally evolved DLBCL in patients with CLL. Clinical trials should always be considered for clonally evolved DLBCL because standard intensive therapy is usually not very effective. Allo-SCT should be attempted if the patient is eligible.

Nonhematological malignancies

Skin cancer

CLL markedly increases the risk and aggressiveness of skin malignancies. Squamous cell carcinoma and basal cell carcinoma (BCC) are increased 5- to 10-fold and have more aggressive biology with increased risk of local invasion and distant metastasis. The risk of melanoma is significantly increased with more aggressive biology and poorer outcome.

Management. Patients need to be educated about limiting ultraviolet radiation exposure and undergoing frequent skin checks with prompt evaluation and management of suspicious lesions. Patients should be seen at least annually by a skilled dermatologist.

Other malignancies

CLL patients are at increased risk of noncutaneous second malignancies, which are a major cause of morbidity and mortality. Patients should minimize high-risk behavior and follow standard cancer-preventative screening guidelines.

KEY POINTS

- CLL is associated with both significant humoral and T-cell-mediated immunodeficiency, leading to an increased risk of infection even for untreated CLL.
- Five percent to 10% of CLL patients have autoimmune complications, the most common being AIHA and ITP.
- Diffuse large B-cell lymphoma and Hodgkin lymphoma are both seen in increased frequency in CLL.
- CLL is associated with an increased incidence of both non-melanoma and melanoma skin cancers that may be more clinically aggressive.

B-cell prolymphocytic leukemia

B-cell prolymphocytic leukemia (B-PLL) is a very rare mature B-cell lymphoid malignancy with a median age at diagnosis of 69 years and equivalent incidence in males and females.

Clinical presentation

Patients with B-PLL usually present with very high ALC (>100,000), splenomegaly that can be massive, and minimal or no lymphadenopathy.

Diagnosis

Diagnosis is suggested by a high percentage (~90%) of lymphocytes with prolymphocytic morphology. These cells are medium sized with large condensed nuclei, a prominent large nucleolus, and a small amount of basophilic cytoplasm without cytoplasmic projections. On flow cytometric analysis, these B-PLL cells are monoclonal B cells that have bright light chain and CD20 expression and usually do not express CD5 or CD23. These features are useful in differentiating primary B-cell PLL from CLL with high levels of prolymphocytes and the leukemic phase of mantle-cell lymphoma. The other considerations in the differential diagnosis are marginal-zone lymphoma and hairy cell leukemia.

Genetic analysis

FISH analysis for t(11;14) should be done to exclude the diagnosis of mantle-cell lymphoma. Approximately 50% of patients have 17p13 deletion/TP53^{mutation} that is associated with poorer responses to chemotherapy regimens.

Treatment

B-PLL is a rare disease with limited data from clinical trials and no standard-of-care therapy. Patients with this disease should be referred to specialized lymphoid malignancy programs for management. Patients without 17p13 deletion/

TP53^{mutation} can respond to CIT regimens similar to those used in the treatment of CLL. Patients with 17p13 deletion/TP53^{mutation} can respond to a combination of anti-CD20 monoclonal antibodies and alemtuzumab. There is very little published data on the use of BCR pathway inhibitors to treat B-PLL.

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