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Molecular basis of hematology

Laura G. Schuettpelz, James R. Cook and Timothy A. Graubert

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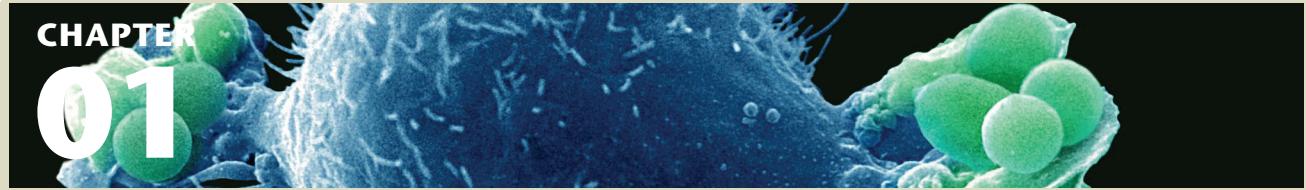
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CHAPTER
01



Molecular basis of hematology

Laura G. Schuettpelz, James R. Cook, and Timothy A. Graubert

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Basic concepts

Advances in recombinant DNA technology over the past 25 years 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 the following 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, and an explanation of the terminology necessary for understanding the role of molecular biology in breakthrough discoveries. Emerging diagnostic and therapeutic approaches in hematology will be 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 nucleo-

tide. 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**.

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 nonhistone proteins that "shield" the DNA from the proteins that activate gene expression.

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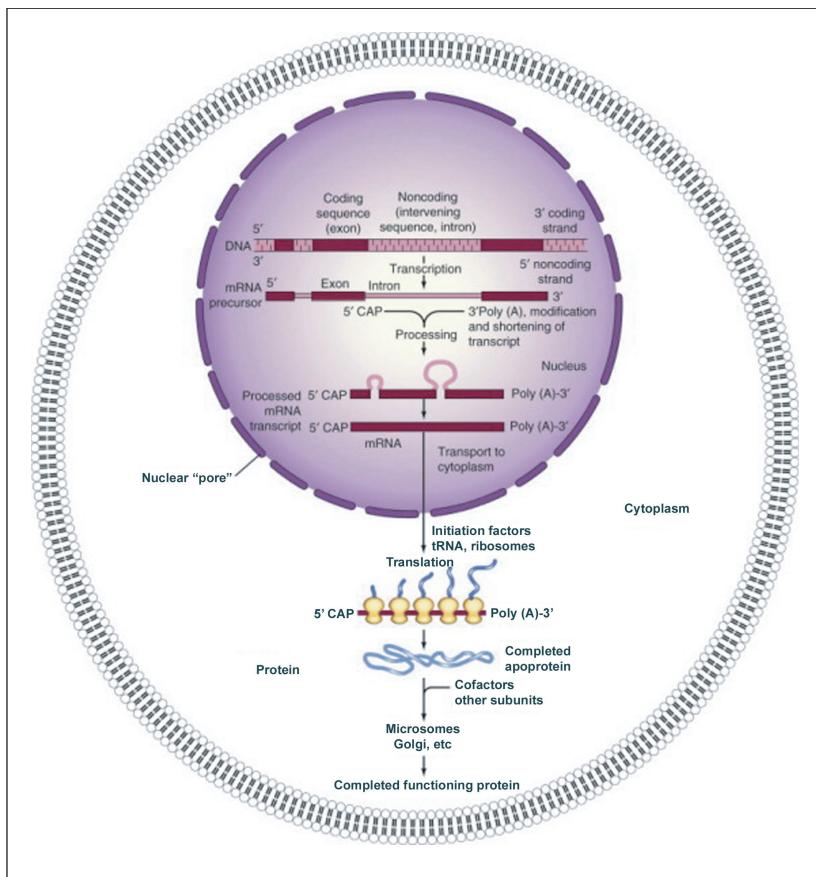


Figure 1-1 Flow of genetic information from DNA to RNA protein. DNA is shown as a double-stranded array of alternating exons (red) and introns (pink). Transcription, posttranscriptional processing by splicing, polyadenylation, and capping are described in the text. The mature transcript passes from the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

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 **premessenger 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. This transcribed mRNA is complementary to the DNA antisense strand. The pre-mRNA contains the sequences of all of the gene's exons and introns. 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 that 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 was thought to be 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 (50-100) and five small nuclear ribonucleic proteins (snRNPs). mRNA splicing is an important mechanism for generating diversity of 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 [ALA] 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. Recently, recurrent mutations in mRNA splicing factors have been identified in patients with myeloid malignancies. The effect of these mutations on splicing has not yet been elucidated. 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.

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 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. Sickle cell disease, however, is an example of a single base-pair change (point mutation) 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 **frameshift 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 locus control region (LCR), which was first and best 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 in 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 is emerging 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 nutrition or drugs 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, the DNA of inactive genes is modified by the addition of methyl groups to cytosine residues. **Methylation** normally occurs throughout the genome. 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. This type of epigenetic modification is performed by enzymes called DNA methyltransferases and is associated with alterations in gene expression and processes, such as X chromosome inactivation, imprinting, and carcinogenesis.

Monzygotic twins accumulate different methylation patterns in the DNA sequences of their somatic cells as they age, increasing phenotypic differences. Lifestyle disparities, especially smoking, result in even greater differences in their DNA methylation patterns. Thus, despite having identical DNA sequences, twins become increasingly dissimilar because of epigenetic changes that result in different expression of their identically inherited genes.

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 of 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*, encoding 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, aberrant methylation may contribute to cancer. For example, in one form of hereditary colorectal cancer, methylation of the promoter region of the *MLH1* gene, whose protein product repairs damaged DNA, results in colon cancer. Likewise, methylation-associated silencing of the DNA repair gene *BRCA1* is associated with breast and ovarian cancers, and hypermethylation of the promoter of the DNA repair gene *MGMT* correlates with improved clinical outcomes in patients with gliomas treated with Temozolamide. Small molecule inhibitors of DNA methyltransferases (eg, 5-azacitidine, decitabine) are used in the treatment of hematologic disorders that are characterized by aberrant DNA methylation (eg, myelodysplastic syndrome [MDS], acute myeloid leukemia [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 are associated with silencing of the cell cycle regulator *p21^{WAF1}*, a gene whose expression is reduced in multiple tumor types. Small molecule inhibitors of the enzyme that removes acetyl groups from histone tails (histone deacetylases) are being tested in a variety of hematologic malignancies, and the histone deacetylase inhibitor Vorinostat is used in the treatment of cutaneous T-cell lymphoma.

Noncoding RNAs

It has been estimated that only approximately 1%-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), 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 a **RISC** (RNA-induced silencing complex). 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. Recent studies suggest that as much as 20% of cellular RNA is regulated by RNA interference. As mediators of gene expression, miRs and other ncRNAs play regulatory roles in development and differentiation, and they also are expressed in a tissue-specific manner. Dysregulation or mutations in ncRNAs are associated with various diseases, including cancer. Increased

expression of miR-21, for example, is associated with poor prognosis in chronic lymphocytic leukemia, and higher expression of miR-29b is associated with clinical response to decitabine in older adults with AML.

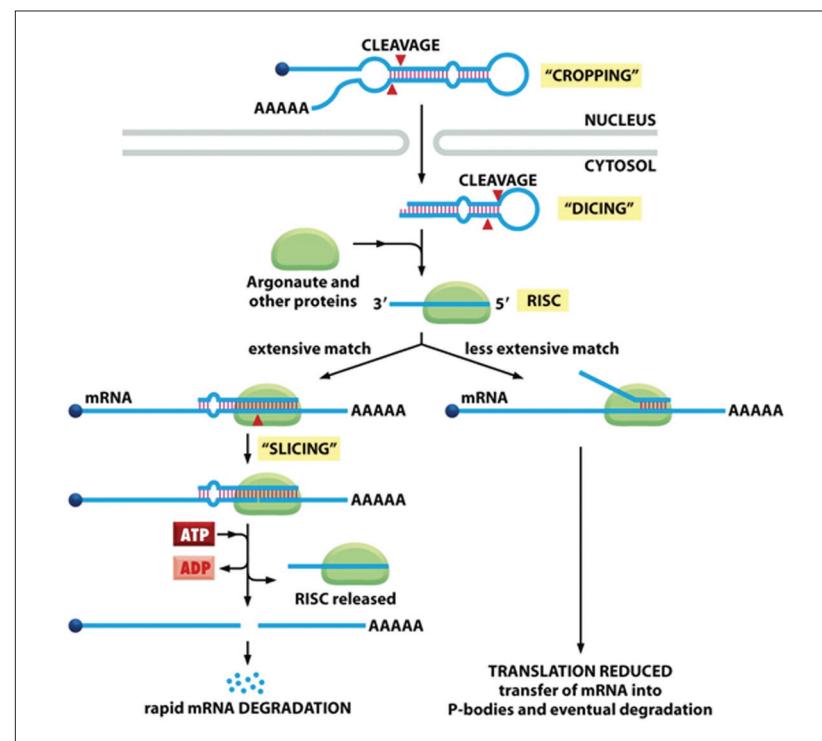
Molecular basis of neoplasia

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 confer a growth or survival advantage to 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

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 Murphy, et al, eds. *Molecular Biology of the Cell*. 5th ed. New York, NY: Garland Science/Taylor & Francis LLC; 2008.



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 for 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 inheritance 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. More recently, loss of just one copy of a gene (“haploinsufficiency”) has been shown to contribute to cancer development (eg, *RPS14* haploinsufficiency in MDS).

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 4–8 bp in double-stranded DNA. These recognition sequences are usually palindromic (ie, 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 ethidium bromide, a chemical that inserts itself between the DNA strands and fluoresces upon exposure to 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 1-3). 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 primers 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 bounded 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 for 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, amplification 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

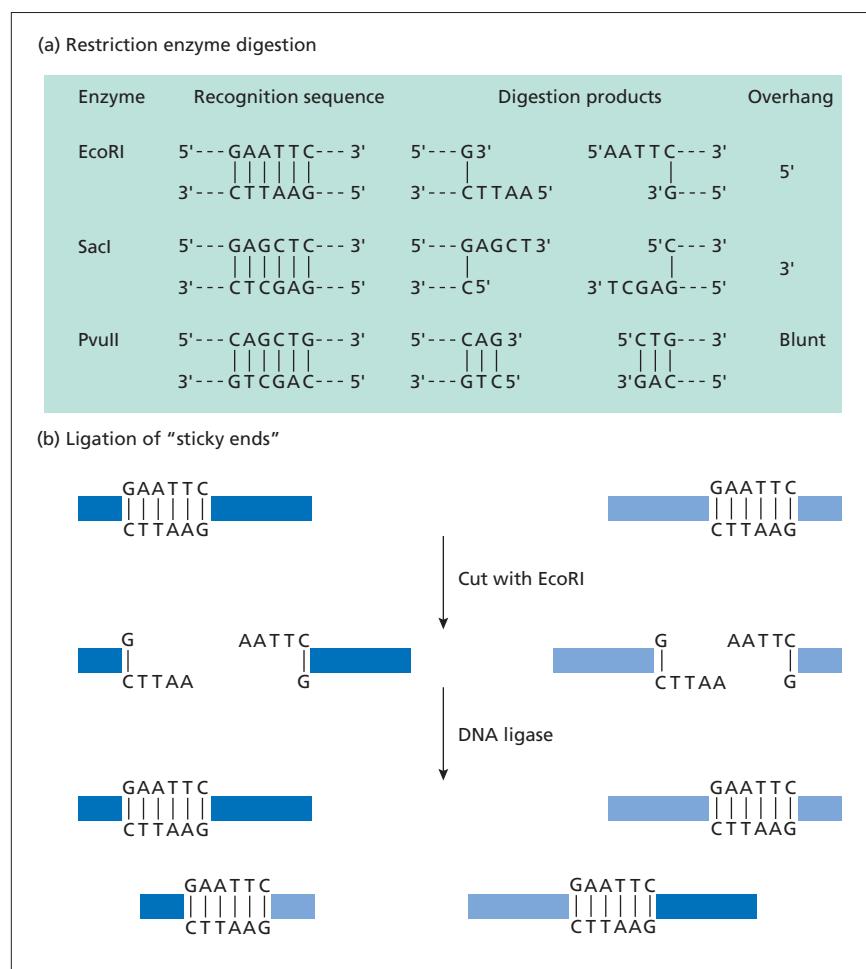


Figure 1-3 Restriction endonuclease digestion. (a) Diagram of typical restriction enzyme recognition sequences and the pattern of cleavage seen upon digestion with that enzyme. (b) Means by which restriction enzyme can be exploited to form recombinant proteins. Digestion of the two fragments with the enzyme *Eco*RI results in four fragments. Ligation with DNA ligase can regenerate the original fragments, but it also can 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 then can be used as a template for generation of recombinant protein in expression vectors.

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 fluorogen tag is released, generating a fluorescent signal (Figure 1-4). Real-time PCR detects the number of cycles when amplification of product is exponential and expresses this as a ratio to standard housekeeping RNA, such as ribosomal RNA or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. This number can be converted to the number of molecules of mRNA present in the test sample. This technique is used widely to measure minimal residual disease 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 can be 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 the 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

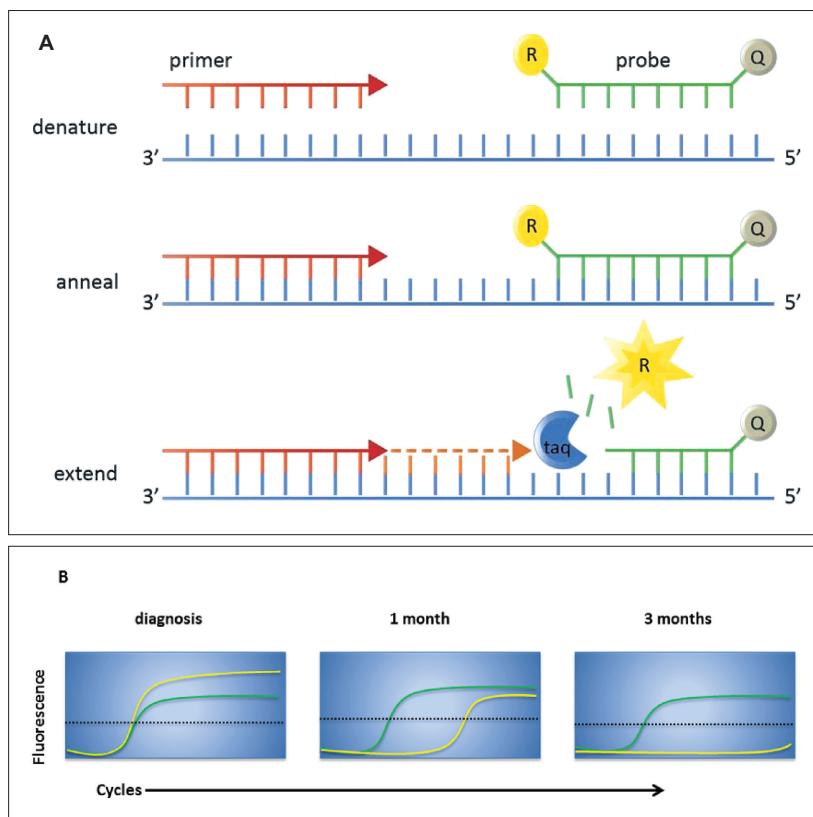


Figure 1-4 Real-time PCR. (a) Sample DNA (or cDNA) is denatured, and target-specific primers are annealed to begin the PCR amplification (shown for one strand). An oligonucleotide probe complementary to a sequence within the PCR product is included in the reaction. The probe contains a fluorophore ('R') 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.

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 reform. 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.

To perform Southern blot analysis, DNA is isolated from peripheral blood, bone marrow, or tumor tissue. The total cellular DNA is then digested with specific restriction enzymes. This results in a wide range of fragments that may be separated by size using agarose gel electrophoresis. Because the DNA will be digested into thousands of fragments, genomic DNA will appear in the gel as a continuous smear. The DNA in the gel is denatured by exposure to alkaline buffer, and the resulting single-stranded species are transferred and fixed to a nitrocellulose or nylon membrane.

Detection of a specific gene fragment requires the use of a **probe**. A probe is a labeled, single-stranded fragment of DNA that is specific to the gene of interest. Probes can be produced from any portion of gene whose sequence is known or that previously has been isolated, such as globin or the immunoglobulin heavy- and light-chain genes. The

denatured, labeled probe dissolved in hybridization solution is incubated with the denatured Southern blot membrane, which contains single-stranded DNA corresponding to the entire cellular DNA. By molecular hybridization, the probe will anneal to complementary sequences within the DNA fixed to the membrane. After the membrane is washed to remove the excess unbound probe and the probe that has hybridized nonspecifically to areas of low-sequence homology, the membrane is exposed to radiographic film or a fluorescence detection system. The resultant image will allow visualization of the DNA fragment or fragments that represent the gene of interest with sequence complementary to the probe (Figure 1-5). Southern blotting may be used to determine whether a gene is present or absent or whether it has been grossly rearranged by deletion, insertion, or recombination.

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,

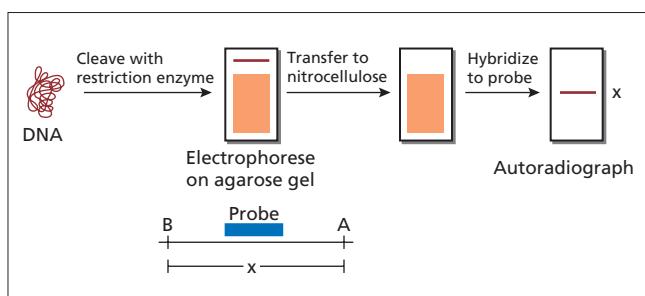


Figure 1-5 Southern blot analysis of DNA. DNA is cleaved with a restriction endonuclease, electrophoresed through an agarose gel, and transferred to nitrocellulose. The probe, as illustrated at the bottom of the figure, lies on a piece of DNA of length x when DNA is digested with an enzyme that cleaves 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.

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 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 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**. Proteins are detected by specific antibodies directed against the protein 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 (APL). 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 minimal residual disease 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 APL. 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 *RAR α* 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) has not only 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 (GWAS), 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** (pUPD), 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, genomewide 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, acute lymphoblastic leukemia (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.

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-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 1-6). 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 for 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). 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 oli-

gonucleotide 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**). Finally, 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.

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.

Proteomics

Proteins are the effectors of most cellular functions. Genetic defects perturb normal cellular functions because they result in changes in the level or the function of the proteins they encode. Many proteins undergo extensive posttranslational 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.

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-assisted laser desorption/ionization time of flight (MALDI-TOF) 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

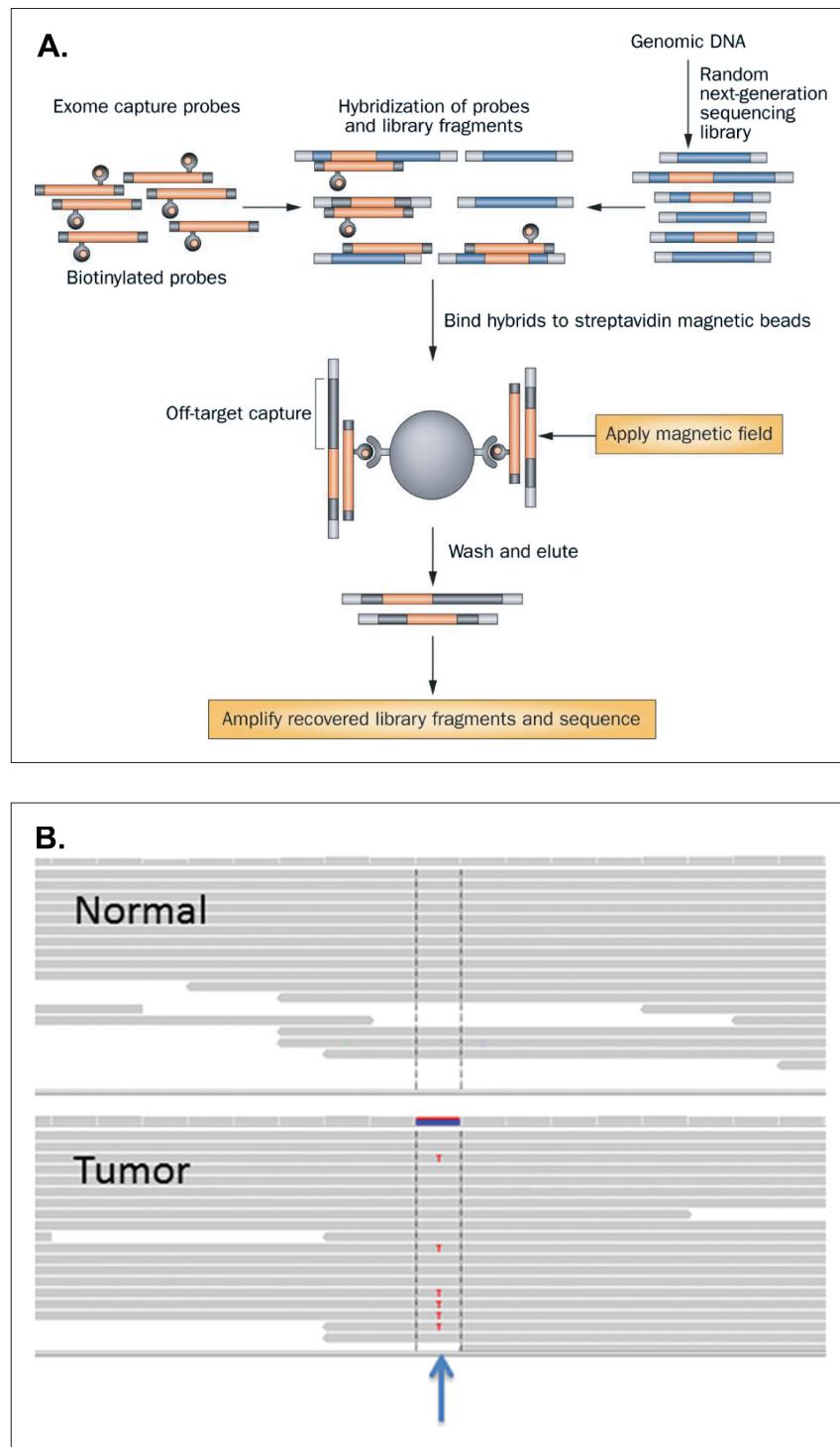


Figure 1-6 Next-Gen sequencing. (a) A library of genomic DNA fragments is shown at the upper right. This library can be used directly for sequencing (whole genome), or can be hybridized with probes (shown in upper left) to enrich for targets in candidate genes or all coding genes (whole exome). Reproduced with permission from Mardis E. Applying next-generation sequencing to pancreatic cancer treatment. *Nat. Rev. Gastroenterol Hepatol.* Vol. 9, pp. 477-486, 2012. (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. *Nature Biotechnology*. “Integrative Genomics Viewer,” Vol. 29, pp. 24-26, 2011). Each grey 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.

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 (GFP) 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 intro-

duced 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 expresses 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.

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 (HPLC) 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 Barts (4 tetramers) on electrophoresis or HPLC, 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, 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%-95% of normal. 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. These deletions can be detected by Southern blot analysis or PCR. The details of this process in lymphocyte ontogeny are further outlined in Chapter 18. Southern blot analysis showing clonal rearrangement of the immunoglobulin κ light chain is illustrated in Figure 1-7.

In recent years, PCR-based approaches have largely replaced Southern blotting in the diagnostic setting because they are more rapid, are less technically demanding, and require less DNA. In addition, PCR studies, unlike Southern blots, can be performed on archived formalin-fixed, paraffin embedded (FFPE) tissue. Modern comprehensive primer sets, such as those developed by the Euroclonality consortium (so-called Biomed-2 primers) can detect clonal Ig and TCR rearrangements with a sensitivity equivalent to Southern blot studies. PCR-based techniques targeting *IGH* and *IGK* loci for B-cell rearrangements and *TCRG* and *TCRB* for T-cell rearrangements are now widely available. PCR clonality studies can be 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. Despite their power, molecular clonality studies should be carefully interpreted in the context of the clinical, morphologic, and immunophenotypic diagnosis. Clonal proliferations

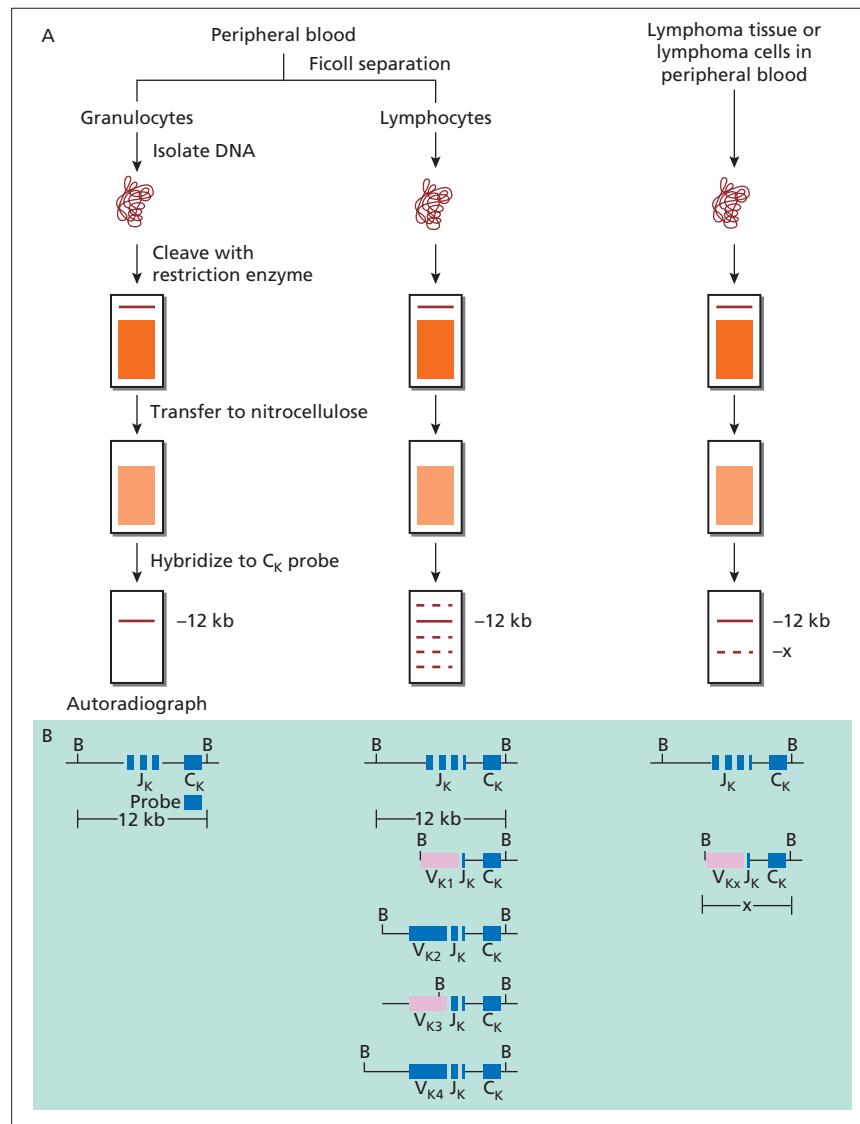


Figure 1-7 Southern blot analysis of κ light-chain rearrangement to establish clonality of populations of B lymphocytes. In (a), the Southern technique and its predicted results in different cell populations are diagrammed; in (b), the molecular configurations of the κ locus are outlined. In the leftmost panel, genomic DNA, as represented in peripheral blood granulocytes, is analyzed. Because all of these cells retain their immunoglobulin genes in the germline configuration, there is a single band on Southern blot analysis corresponding to germline κ locus. In the middle panel, polyclonal lymphocytes also show only a germline band on Southern analysis. However, this does not reflect the fact that the cells do not have rearrangement of the κ locus. It is instead a reflection of the fact that there are insufficient cells of any one clone to be detectable as a discrete band on Southern analysis. Because a significant fraction of the cells will retain one κ locus that is unarranged, a germline

band is detectable. (In normal peripheral lymphocytes, this band also partly reflects T cells, which of course do not rearrange their immunoglobulin gene loci.) The rightmost panel diagrams the Southern pattern produced by monoclonal tumor cells bearing a κ light chain. All of the cells of the population will have an identical rearrangement of the κ genes that will be apparent as a rearranged band on Southern blot analysis. The figure demonstrates the pattern of cells in which one κ locus has rearranged. If both chromosomes are rearranged, then there will be two rearranged bands and the germline band will no longer be present. Reproduced with permission from Berliner N. Use of molecular techniques in the analysis of hematologic disease. In: Hoffman R, Benz E, Silberstein L, Heslop H, Weitz J, Anastasi J, eds. *Hematology: Basic Principles and Practice*. 6th ed. Philadelphia, PA: Saunders Elsevier, Inc.; 2013: 12.

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. 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.

Identification of cryptic translocation in pediatric acute lymphoblastic leukemia: prognostic significance

As many as 20% of children with pre-B-cell ALL have a translocation that fuses the *RUNX1* gene on chromosome 21 with the *ETV6* gene on chromosome 12 in the t(12;21) translocation. Both of these genes encode transcription factors that regulate hematopoietic differentiation. Although this translocation is common, it is rarely seen by standard cytogenetic techniques. It, however, can be routinely identified with FISH or with RT-PCR using specific *ETV6-RUNX1* probes. The identification of this translocation is important because it has been associated with a favorable outcome in pediatric ALL.

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 the *NPM1*, *FLT3*, and *CEBPA* genes. *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 [ITD]) 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. In recent years, numerous other recurrent mutations also have been identified that appear to be prognostic in normal karyotype AML. In particular, mutations in *DNMT3A* have emerged as powerful predictors of poor prognosis in AML using multivariate analysis. 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 may

allow for improved prognostic assessment and treatment selection based on testing a larger number of recurring mutations in myeloid neoplasms, including AML, myelodysplastic syndromes, and myeloproliferative neoplasms.

Minimal residual disease monitoring

The development of PCR has markedly increased the sensitivity of tests available for the monitoring of minimal residual disease 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 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 level 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 Imatinib 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 10^5 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.

Expression profiling: applications to diagnosis and treatment

Gene expression microarray studies have yielded important new insights into leukemia pathogenesis and may facilitate the development of therapeutic interventions. Early studies demonstrated that the distinction between ALL and AML could be made with 100% accuracy on the basis of gene expression profiles alone. Later studies demonstrated that distinct molecular subtypes of ALL and AML could be discriminated based on expression profiling. For example, the application of expression microarray technology to the evaluation of infant ALL with rearrangement of the *MLL* gene located at chromosome 11q23 demonstrated a molecular signature with features of both myeloid and lymphoid lineages. In addition, this work identified the *FLT-3* gene as one of the genes distinguishing infants with *MLL*-associated ALL from those with non-*MLL*-rearranged, pre-B-cell ALL. Microarray technology also may accurately delineate subtypes of a heterogeneous disorder, such as pediatric ALL. For example, hierarchical clustering has been used to distinguish pediatric ALL subtypes: *E2A-PBX*, *MLL*, T-ALL, hyperdiploid, *BCR-ABL1*, and *ETV6-RUNX1*.

Microarray studies are facilitating 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 (DLBCL): germinal center and activated B-cell types, which carry favorable and unfavorable prognosis, 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 (GVHD) in patients who receive transplantations from serologically 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 GVHD

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, 17–20 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.

Glossary

alleles Alternative forms of a particular gene.

allele-specific oligonucleotide 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.

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.

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.

ChIP-Seq A combination of chromatin immunoprecipitation followed by Next-Gen sequencing used to identify protein–DNA interactions.

chromatin Complex of genomic DNA with histone and nonhistone proteins.

chromosome Large linear DNA structures 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 Portion of the gene contained within exons that encodes for 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.

cytogenetics 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 *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.

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 All protein coding portions of genes (exons) in the genome.

exon Portion of the structural gene that encodes for protein.

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

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 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 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 Process controlling the timing and level of expression of a gene.

genetic code System by which DNA encodes for specific protein 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.

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.

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

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

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.

knockout mouse 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 analysis 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 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.

microRNA 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.

Next-Gen sequencing Massively parallel sequence production from single-molecule DNA templates.

noncoding sequences DNA sequences that do not directly encode for protein.

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 Technique of nucleic acid analysis via association of complementary single-stranded species.

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

oligonucleotide Short single-stranded DNA species, usually composed of 15–20 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.

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 Phenotypically silent mutation in DNA that is transmitted from parent to offspring.

premessenger RNA Unprocessed primary RNA transcript from DNA, including all introns.

probe Fragment of DNA derived from a genetic locus that can be labeled, denatured, and used to analyze that gene.

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

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

pyrimidine 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 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)

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 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 Ribonuclear protein complexes that bind to mRNA and mediate its translation into protein by reading the genetic code.

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

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 polymerase II Enzyme-mediating transcription of most structural genes.

RNA-seq Next-Gen sequencing using RNA templates.

silencer *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.

siRNA Small interfering RNAs that act in concert with large multiprotein RISC complexes 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 Process by which intron sequences are removed from pre-mRNAs.

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

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

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

transcription factor 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 Process by which protein is synthesized from an mRNA template.

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

tumor suppressor Genes that promote tumor development when deleted or inactivated.

uniparental disomy Occurs when 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.

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

zinc-finger 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.

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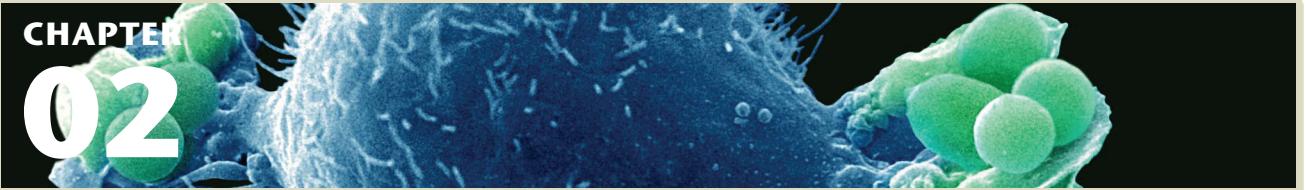
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CHAPTER
02



Consultative hematology I: hospital-based and selected outpatient topics

Donald M. Arnold, Adam C. Cuker, and Cindy Neunert

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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. 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 house staff, fellows, students, and the patient and family. Group meetings are often invaluable for the management of complex patients. A commitment to effective communication ensures maximal compliance with recommendations and the highest quality of multidisciplinary patient care.

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

Consultation for surgery and invasive procedures

Conflict-of-Interest disclosure: Dr. Arnold: consultancy: Amgen; GlaxoSmithKline; Hoffman-LaRoche. Research funding: Amgen; GlaxoSmithKline; Hoffman-LaRoche. Honoraria: Amgen. Membership on board of directors of advisory committee: Amgen; GlaxoSmithKline. Dr. Cuker: consultancy: Baxter; Bayer. Research Funding: Baxter; Bayer; Stago. Dr. Neunert declares no competing financial interest.

Off-label drug use: Dr. Arnold: Cyclophosphamide and rituximab for use in antiphospholipid antibody syndrome. Dr. Cuker: Desmopressin for use in platelet function disorders; Desmopressin, Recombinant FVIIa, Prothrombin complex concentrate, Activated prothrombin complex concentrate, and Tranexamic acid for use in surgical bleeding; Conjugated estrogens for use in uremic bleeding; Angiotensin converting enzyme inhibitors and angiotensin receptor blockers for use in post-renal transplant erythrocytosis; Rituximab for use in CD20+ post-transplant lymphoproliferative disorder.

Clinical case

A 62-year-old man with recently diagnosed pancreatic cancer is scheduled to undergo a Whipple procedure. Four weeks ago, he suffered an above-the-knee, right lower extremity, deep venous thrombosis (DVT) for which he is currently on twice-daily low-molecular weight heparin (LMWH). You are consulted to assist with perioperative anticoagulation. In light of the patient's recent DVT, you judge his thrombotic risk to be high. Because of the risk of tumor progression, the surgery cannot be delayed. You advise the patient to administer his last dose of

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 second-hand information
Communicate as briefly as appropriate	Compliance is optimized when the consultant addresses specific questions with five 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. Ten commandments for effective consultations. *Arch Intern Med.* 1983;143:1753-1755; and Sears CL, Charlson ME. The effectiveness of a consultation. Compliance with initial recommendations. *Am J Med.* 1983;74:870-876.

Clinical case (continued)

LMWH 24 hours before surgery. You also recommend initiation of intermittent pneumatic compression (IPC) in the immediate postoperative period and resumption of LMWH 48 hours after surgery provided that adequate hemostasis has been achieved.

Perioperative management of antithrombotic therapy

The perioperative management of patients taking antiplatelet or anticoagulant drugs is based on (i) an assessment of the patient's risk for thromboembolism and (ii) an assessment of risk for perioperative bleeding. These considerations are used to determine whether antithrombotic therapy should be interrupted prior to surgery and, if so, whether bridging anticoagulation should be considered. 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%-10%, and <5%, respectively. Individuals with mechanical mitral valves, atrial fibrillation and CHADS₂ scores of 5 or 6, recent stroke or venous thromboembolism (VTE) are considered high risk. Those with atrial fibrillation and CHADS₂ scores of 0-2 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. This risk must be weighed against the risk of surgical

hemorrhage. An assessment of hemorrhagic risk should take into account the propensity for bleeding associated with both the procedure and antithrombotic agent in question. Guidelines on the perioperative management of antithrombotic medications recently were updated by the American College of Chest Physicians (ACCP) (<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 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. In patients requiring minor dental procedures, warfarin may be continued with coadministration of an oral antifibrinolytic agent or warfarin may be stopped 2-3 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. Like warfarin, the new oral anticoagulants, dabigatran, rivaroxaban, and apixaban, must be discontinued before major surgery. 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 warfarin, relies on an assessment of the individual 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, those at low risk of cardiovascular events

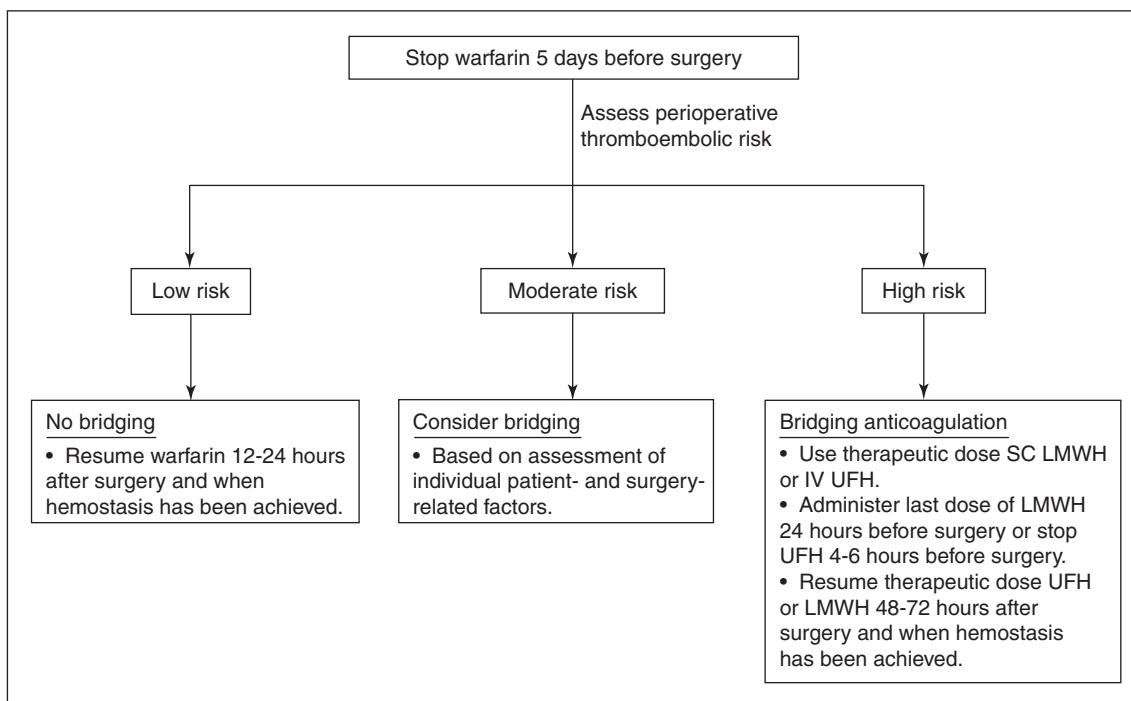


Figure 2-1 Approach to perioperative management of patients on warfarin undergoing major surgery. Management should be informed by an individualized assessment of host- and surgery-related risk factors for perioperative thromboembolism and hemorrhage as well as patient values and preferences. LMWH =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

should discontinue aspirin 7–10 days before surgery. Aspirin should be continued in patients judged to be at moderate or high risk. Patients who require coronary artery bypass grafting (CABG) 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, surgery should be deferred, if possible, during the period of highest risk for in-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.

Preoperative assessment of bleeding risk

Bleeding risk is related to both surgical and host factors. 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, 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 is the most important tool for assessing the risk of operative bleeding. The 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. 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.

If the history or physical examination is suggestive of a bleeding diathesis, a hemostatic laboratory evaluation should be performed before the patient is cleared for surgery. Initial 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 based on

Table 2-2 General risk stratification for patients undergoing nonorthopedic surgery.

Risk category	Risk of VTE (without prophylaxis)	Type of surgery			
		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
Low	~1.5%	Rogers score 7-10	Caprini score 1-2	Caprini score 3-4	Spinal surgery for nonmalignant disease
Moderate	~3.0%	Rogers score >10	Caprini score 3-4	Caprini score 5-6	<ul style="list-style-type: none"> • Gynecologic noncancer surgery • Cardiac surgery • Most thoracic surgery • Spinal surgery for malignant disease
High	~6.0%	NA	Caprini score ≥5	Caprini score 7-8	<ul style="list-style-type: none"> • Bariatric surgery • Gynecologic cancer surgery • Pneumonectomy • Craniotomy • Traumatic brain injury • Spinal cord injury • Other major trauma

Adapted from ACCP guidelines.

VTE = venous thromboembolism; NA = not applicable.

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 $50 \times 10^9/\text{L}$ 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 $100 \times 10^9/\text{L}$. Hemostatic laboratory testing is neither cost effective nor informative in patients without a history suggestive of a bleeding disorder.

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, such as factor deficiency, von Willebrand disease, or a platelet function disorder; 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.

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 fibrinolysis, or the use of antiplatelet agents, heparin, or other anticoagulants. 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. Significant abnormalities of any of these parameters suggest a systemic hemostatic defect, which may require specific hemostatic therapy. For example, a prolonged PT or aPTT may suggest deficiency of one or more clotting factors. A mixing study should be performed to exclude an inhibitor and individual factor levels should be assessed to guide replacement 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 $50 \times 10^9/\text{L}$ ($100 \times 10^9/\text{L}$ for organ- or life-threatening bleeding), respectively. Hypothermia and acid-base disturbances should be corrected.

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, respectively. 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 5–7 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, 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%–40% reduction in surgical blood loss without an increased risk of thromboembolism. Aprotinin, an antifibrinolytic agent derived from bovine lung, was shown to be associated with an excess of kidney injury and death in a randomized controlled trial and observational studies and subsequently has been suspended from the market in some jurisdictions.

Recombinant factor VIIa (rFVIIa) is approved for the treatment of patients with congenital hemophilia A or B and inhibitors and patients with acquired hemophilia in the United States. In the European Union, it also is indicated for patients with congenital factor VII deficiency and Glanzmann's thrombasthenia. The majority of rFVIIa usage is off-label, however, especially for the management of perioperative bleeding. Two small randomized controlled trials, one in men undergoing retropubic prostatectomy and the other in patients with bleeding after cardiac surgery, suggested that the use of rFVIIa was associated with reduced blood loss and need for transfusion. In contrast, controlled trials have shown rFVIIa 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 rFVIIa. In a meta-analysis of 35 randomized controlled trials of rFVIIa for unapproved indications, the overall rate of thromboembolism in rFVIIa-treated subjects was 9.0%. The rate of arterial, but not venous, events was higher in subjects receiving rFVIIa, particularly among those 65 years and older. The indiscriminant use of

rFVIIa 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 rFVIIa.

Prothrombin complex concentrates (PCCs) are plasma-derived concentrates of the vitamin K-dependent clotting factors. These products are approved for the treatment of hemophilia B, although they are more commonly used for emergent reversal of warfarin-induced coagulopathy. 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 PCC and APCC 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, is composed of purified, virally inactivated human fibrinogen, human thrombin, and aprotinin. Although randomized clinical trial data and evidence-based guidelines are lacking, fibrin sealant is used in cardiac surgery, urologic procedures, orthopedic surgery, dental procedures, trauma, and neurosurgery, where it is administered to seal dural leaks and repair otic ossicles and bony defects. It also has been used on the liver surface following orthotopic liver transplantation to augment local hemostasis.

Perioperative red blood cell transfusion

Red blood cell (RBC) transfusion is common in the perioperative setting. Depending on the patient population and threshold for transfusion, rates of RBC transfusion after CABG range from 40%–90%. Similar rates have been reported following total hip and knee arthroplasty.

The threshold for RBC transfusion in surgical patients has changed over time. For many years, patients generally were transfused to maintain a hemoglobin of 10 g/dL or higher. Efforts to contain costs as well as concerns regarding adverse effects of RBC transfusion, however, have forced a reexamination of transfusion practices. In addition to the risks of blood transfusion common to all clinical settings (transmission of bloodborne pathogens, transfusion reactions, circulatory overload), a recently published longitudinal observational study suggested that surgical patients receiving 1 unit of intraoperative RBCs had a significantly higher rate of unadjusted mortality and postoperative wound, renal, infectious, and pulmonary complications than those not transfused intraoperatively. Although these data require confirmation in a controlled trial, they sound a cautionary note regarding the potential harms of unnecessary transfusion.

The findings of several large clinical trials show that, in general, a liberal RBC transfusion strategy does not improve clinical outcomes, and a restrictive transfusion strategy is as safe, if not safer. Thus, recent guidelines suggest that in adult and pediatric intensive care unit (ICU) patients, transfusion should be considered at hemoglobin concentrations of 7 g/dL or less, and in postoperative surgical patients, transfusion should be considered at a hemoglobin concentration of 8 g/dL or less, or symptoms (chest pain, orthostatic hypotension or tachycardia unresponsive to fluid resuscitation, or congestive heart failure). The decision 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.

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. Use of autologous blood peaked in the 1980s and early 1990s in response to the AIDS epidemic. With routine nucleic acid testing (NAT), the risk of contracting HIV or hepatitis C from a unit of blood in the United States is currently approximately 1 in 1.5 million. Although PAD all but eliminates this risk, autologous donation is associated with its own drawbacks. PAD is much more expensive than allogeneic blood and, as with any blood product, is subject to bacterial growth during liquid storage, volume overload, hemolysis from improper handling of stored units, and clerical error, resulting in inadvertent administration of an allogeneic product. Moreover, a meta-analysis demonstrated that patients who underwent PAD were threefold more likely to receive a blood transfusion than those who did not. Both a higher incidence of anemia due to PAD and a more liberal transfusion policy when autologous blood is available were thought to be responsible for this difference. The donation process itself carries a small risk of adverse events, including hypotension, orthostasis, arrhythmias, and ST-T wave changes and therefore is contraindicated in patients with recent acute coronary syndrome or cerebral vascular accident, heart failure, or aortic stenosis. On the basis of the aforementioned limitations of autologous donation and the improved safety of allogeneic products, it is recommended that PAD be restricted to healthy individuals requiring blood-intensive surgeries in which the likelihood of blood loss in excess of 500–1,000 ml is at least 5%–10%.

Prevention and treatment of postoperative venous thromboembolism

VTE is a common and potentially lethal complication of surgery. In the prophylaxis era, the incidence of fatal pulmonary embolism (PE) was estimated to be 0.1%–0.8% in patients undergoing elective general surgery, 2%–3% in

patients having elective total hip replacement, and 4%–7% in patients undergoing hip fracture surgery. These rates are reduced significantly with modern thromboprophylaxis but remain unacceptably high, causing 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 >30 minutes, 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 nonorthopedic surgery is shown in Table 2-2. 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 40–60 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 surgery as well as those age 40–60 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 ambulation, lower extremity IPC, graduated compression stockings, and pharmacologic prophylaxis with low dose UFH, LMWH, fondaparinux, and oral anticoagulation. In general, early ambulation alone is recommended for very low-risk nonorthopedic surgery patients; mechanical prophylaxis, preferably with IPC, for low-risk patients; pharmacologic or mechanical prophylaxis for moderate-risk patients; and a combination of pharmacologic and mechanical prophylaxis for high-risk patients (<http://chestjournal.chestpubs.org>). An important exception is patients judged to be at high risk for bleeding, in whom 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 generally are 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 are all reasonable options for patients undergoing hip fracture surgery. Any of these agents, as well as the new oral anticoagulants, dabigatran, apixaban, and rivaroxaban, 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 10–14 days after major orthopedic surgery. Extended prophylaxis for 4–5 weeks should be considered after major orthopedic surgery and after major abdominal or pelvic surgery for cancer.

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 12–24 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, and rivaroxaban should be avoided in patients with renal failure.

When VTE occurs postoperatively, the consultant may be asked for treatment recommendations. For most low-risk procedures, full anticoagulation can be initiated safely within 12–24 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 should be made for retrieval of the filter. In general, by the third postoperative day after uncomplicated surgery, the risks of anticoagulation are comparable to those in nonsurgical patients and longer acting anticoagulants, such as LMWH and warfarin, may be initiated in stable patients. 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 cancer or antiphospholipid syndrome.

Key points

- 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.

Key points (continued)

- A focused medical history is the most important tool for assessing 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.
- Recent guidelines suggest that RBC transfusion should be considered in surgical patients with a hemoglobin concentration of 8 g/dL or less or symptomatic anemia. 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.
- 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 anticoagulation and thrombolysis must be carefully considered.

Common inpatient consultations

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

Thrombocytopenia

Clinical case

A 57-year-old man is in the ICU following complications from abdominal surgery seven days ago. He is intubated and mechanically ventilated. He is requiring vasopressor medications to maintain his blood pressure and is receiving broad spectrum antibiotics. You are consulted because his platelet count is $30 \times 10^9/L$. His left leg and several toes are a dusky color and one patch of skin around his left ankle is gangrenous. The PT and aPTT are prolonged and both correct to within the normal range when the test is repeated using mixed normal plasma. The D-dimer and fibrinogen degradation products are elevated. The fibrinogen concentration is 0.8 g/L. You suspect he has DIC with thrombotic complications and recommend full-dose UFH with careful monitoring.

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 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%-68% of patients on admission to the ICU and developed in 13%-44% during their ICU stay. The main challenges in the management of hospitalized patients with thrombocytopenia are to identify the underlying cause and to 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 >1 mechanism of thrombocytopenia (eg, immune thrombocytopenia [ITP] may be caused by both platelet destruction and platelet underproduction). 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): (i) exclude thrombocytopenic emergencies, (ii) examine the blood film, (iii) consider the clinical context, (iv) assess the degree of thrombocytopenia,

(v) establish the timing of thrombocytopenia, and (vi) assess the patient for signs of bleeding.

Exclude thrombocytopenic emergencies

Any thrombocytopenic condition could become an emergency if it is severe and serious bleeding occurs (eg, intracranial hemorrhage in a patient with severe ITP). But some thrombocytopenic disorders are emergencies in themselves because of their associated risk of significant morbidity and mortality if not promptly recognized and managed. These include drug-induced immune thrombocytopenia (DITP), heparin-induced thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), sepsis and DIC, catastrophic antiphospholipid antibody syndrome (CAPS), and post-transfusion purpura (PTP). Hematological emergencies such as acute leukemia also should be considered when not only the platelets but also red and white cells are abnormal or when other signs or symptoms are present. These diagnoses should be considered initially for any patient with thrombocytopenia.

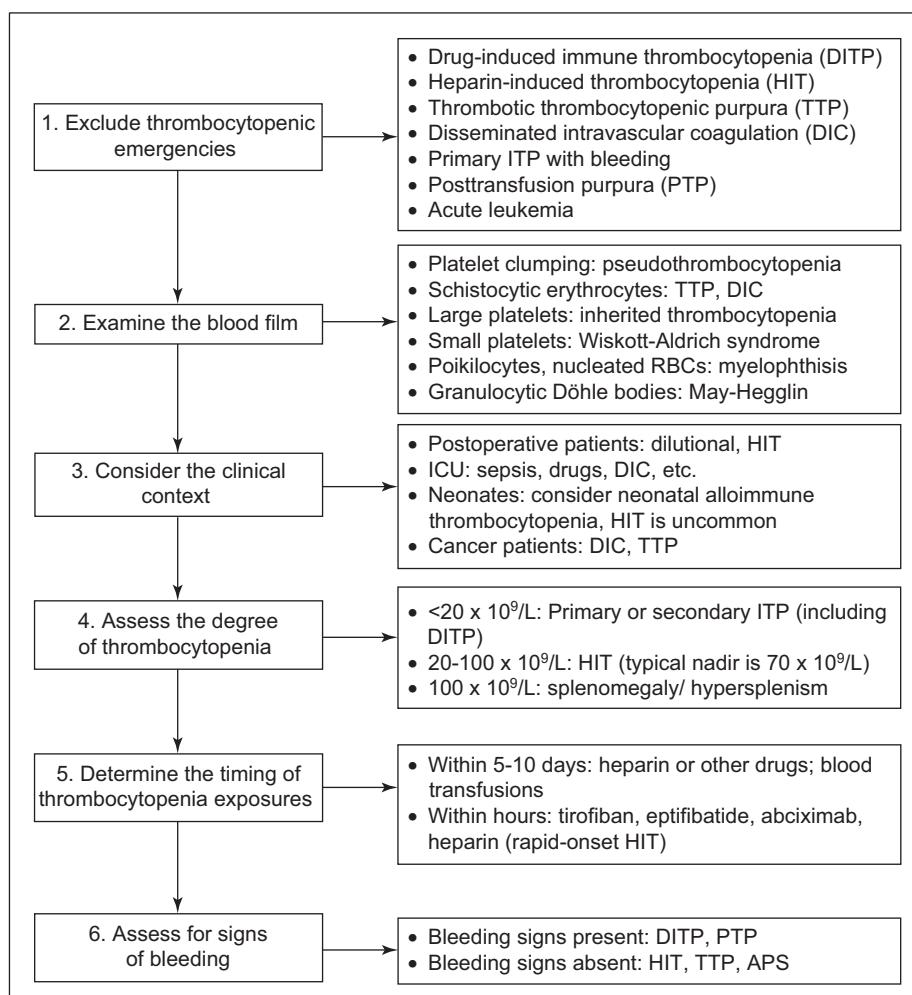


Figure 2-2 Practical approach to the patient with thrombocytopenia. Adapted with permission from Arnold DM, Lim W. A rational approach to the diagnosis and management of thrombocytopenia in the hospitalized patient. *Semin Hematol*. October 2011;48(4): 251-258.

Drug-induced immune thrombocytopenia and heparin-induced thrombocytopenia

DITP is characterized by severe thrombocytopenia and may be associated with serious bleeding complications. DITP is an idiosyncratic reaction caused by drug-dependent platelet-reactive antibodies that cause rapid platelet clearance (see Chapter 10). An expanded list of drugs and the level of evidence for their association with thrombocytopenia has been reported online (<http://www.ouhsc.edu/platelets>). This list includes approximately 150 drugs with a definite or possible association with DITP. A shortened list has been produced by combining clinical and laboratory criteria for the diagnosis of DITP (Table 2-3). Classic DITP reactions, such as quinine-induced DITP, result in thrombocytopenia that occurs 5-10 days after first exposure to the drug. The glycoprotein IIbIIIa inhibitors abciximab and eptifibatide can cause thrombocytopenia within hours of the first drug exposure. Heparin-induced thrombocytopenia (HIT) is a distinct clinical syndrome associated with thrombosis rather than bleeding. Without proper treatment, up to 55% of patients develop thrombosis and approximately 5%-10% of patients will die as a result of thrombotic complications.

Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome

TTP and hemolytic uremic syndrome (HUS) are thrombotic microangiopathies characterized by microangiopathic hemolytic anemia and thrombocytopenia. The clinical manifestations of these disorders overlap, however; patients with TTP often have neurological complications, whereas renal impairment predominates in HUS (see Chapter 10). With proper treatment, survival of TTP patients is 85%. Management requires early institution of daily plasma exchange with 1.0-1.5 plasma volumes; without it, survival drops to 10%. Corticosteroids and rituximab have been used successfully to treat patients with relapsed or refractory TTP and splenectomy has been shown to reduce the rate of relapse in some high-risk patients. A deficiency of ADAMTS13 (A Disintegrin And Metalloproteinase with a Thrombo Spondin type 1 motif), a vWF-cleaving protease, is associated with the development of congenital TTP and some forms of acquired TTP. Patients with HUS often do not require plasma exchange. 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. The atypical form of HUS occurs without a diarrheal prodrome (atypical or diarrhea-negative HUS) and is associated with a higher incidence of end-stage

Table 2-3 Drugs that fulfilled all clinical and laboratory criteria for drug-induced immune thrombocytopenia.

Testing for drug-dependent platelet antibodies confirmed by >1 laboratory	Testing for drug-dependent platelet antibodies confirmed by 1 laboratory
Quinidine	Acetaminophen
Quinine	Amiodarone
Trimethoprim/sulfamethoxazole	Amlodipine
Vancomycin	Ampicillin
Penicillin	Cephalexin
Rifampin	Ciprofloxacin
Carbamazepine	Diazepam
Ceftriaxone	Ethambutol
Ibuprofen	Eurosemide
Mirtazapine	Gold
Oxaliplatin	Haloperidol
Suramin	Lorazepam
Abciximab	Naproxen
Tirofiban	Phenytoin
Eptifibatide	Piperacillin
Heparin	Ranitidine
	Rosiglitazone
	Roxifiban
	Sulfisoxazole
	Tranilast

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 factor B and C3 have been described. Recent data suggest that complement inhibition with the monoclonal antibody eculizumab that targets C5 may be beneficial for some patients with atypical HUS.

Disseminated intravascular coagulation and sepsis

DIC occurs in critically ill patients in the setting of a serious underlying disease, such as sepsis, classical meningococcemia, 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 unimpeded thrombin generation as a result of an imbalance in the normal procoagulant and anticoagulant pathways. 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 demonstrating thrombocytopenia, fibrinogen degradation products, elevated D-dimers, prolongation of the PT and aPTT, decreased fibrinogen concentration and decreased protein C concentration.

A significant reduction in the level of fibrinogen may indicate DIC even if it does not result in hypofibrinogenemia. Fragmentation of RBCs may be seen on the peripheral blood film. DIC is a dynamic process requiring repeated measurements of hemostasis and careful clinical monitoring. A scoring system, incorporating clinical and laboratory indices, was developed by the International Society for Thrombosis and Hemostasis to help with the diagnosis of DIC.

Guidelines and consensus statements for the management of DIC highlight the importance of treating the underlying condition even though this can be challenging. Transfusions of platelet concentrates generally should be reserved for patients with a platelet count below $50 \times 10^9/L$ who have or who are at high risk of bleeding. Evidence is lacking to support the use of prophylactic platelet transfusions; however, platelet transfusion may be reasonable in the context of worsening thrombocytopenia. Similarly, plasma transfusions generally are reserved for patients with an increased PT and bleeding, and cryoprecipitate or fibrinogen concentrates are indicated for patients with severe hypofibrinogenemia (fibrinogen $<100 \text{ mg/dL}$). Prophylactic doses of UFH or LMWH is 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; neither recombinant antithrombin concentrate nor recombinant tissue factor pathway inhibitor have demonstrated a benefit. Initial clinical trials with activated protein C concentrates were promising; however, follow-up studies showed no benefit and raised concerns about bleeding risk. As a result, activated protein C concentrates have been withdrawn from the market.

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 three 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 one organ or tissue; and (iv) laboratory confirmation of the presence of antiphospholipid antibodies (lupus anticoagulant; or anti-cardiolipin or anti- β -2-glycoprotein I antibodies). A registry of patients with CAPS has provided important information on diagnosis and management (<http://www.med.ub.es/MIMMUN/FORUM/CAPS.HTM>). Infection is the most commonly identified precipitant, but other triggers such as trauma, withdrawal of anticoagulation, and neoplasia also

have been described. Approximately 40% of patients with CAPS have no obvious underlying cause. Mortality can be up to 50%. Anticoagulation is the mainstay of therapy with or without high-dose corticosteroids. The highest rates of response have been achieved with the combination of anticoagulation, corticosteroids, and plasma exchange. Intravenous immunoglobulin (IVIg), cyclophosphamide, and rituximab have been used with some success.

Posttransfusion purpura

PTP is a syndrome characterized by severe thrombocytopenia and bleeding that develops 7-10 days after the transfusion of RBCs or platelet 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 (HPA)-1a. The incidence of PTP is estimated at 1-2 per 10,000 transusions, and appears to be less common with leukodepleted 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.

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 at 37°C usually resolves the platelet clumping. The blood film also allows for morphological assessment of erythrocytes and leukocytes, which may provide important clues to the underlying diagnosis: Fragmented RBCs raise the possibility of TTP or DIC; poikilocytes or nucleated RBCs may reflect a myelophthusic process; abnormal leukocytes may indicate a hematologic malignancy or myelodysplasia; toxic granulation of neutrophils are seen in sepsis; and neutrophilic inclusions known as Döhle bodies are associated with hereditary forms of thrombocytopenia, such as the May-Hegglin anomaly.

Consider the clinical context

The clinical context in which the thrombocytopenia developed is an important clue to the underlying diagnosis. Thrombocytopenia is expected after major surgery or in the context of massive transfusion (15-20 units of RBCs) because of dilution or consumption; thrombocytopenia is a common occurrence among critically ill patients; patients with underlying malignancies are prone to DIC or thrombotic

microangiopathies; and patients with liver disease may have chronic thrombocytopenia, which may be exacerbated by their acute illness. Secondary ITP may occur in the setting of HIV or hepatitis C. 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 in patients admitted to the ICU

Approximately 40% of critically ill patients will have thrombocytopenia; however, the frequency varies based on case mix. In a systematic review of medical, surgical, and mixed ICU studies, prevalent thrombocytopenia (on ICU admission) occurred in 8.8%-67.6% of patients and incident thrombocytopenia (occurring during the course of the ICU stay) occurred in 13.1%-44.1% of patients. Thrombocytopenia was an independent risk factor for mortality. The association between thrombocytopenia and bleeding remains uncertain in this population.

HIT in the ICU

The frequency of HIT in ICU patients is 0.3%-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 occurring by days 1-3 is expected due to consumption and dilution; however, delayed platelet count recovery beyond day 7, worsening thrombocytopenia between days 5 and 14, or the development of new thrombosis in an already thrombocytopenic patient may indicate HIT. Furthermore, testing for anti-PF4-heparin antibodies lacks specificity and may lead to false-positive results in critically ill patients; thus, functional platelet-activation tests such as the serotonin release assay should be used to confirm the diagnosis. Treatment of HIT requires anticoagulation with a nonheparin alternative.

Thrombocytopenia in patients on anticoagulation

Although thrombocytopenia can increase the risk of bleeding, it does not protect against thrombosis. Anticoagulation in thrombocytopenic patients with cancer is likely to be safe for most patients with platelet counts above $30 \times 10^9/L$; however, high-quality studies are lacking. The need for anticoagulation in patients with thrombocytopenia is most compelling in antiphospholipid antibody syndrome, HIT, and DIC when thrombosis predominates. The need for anticoagulation may be an indication to treat the

underlying thrombocytopenia when possible. Lowering the intensity of warfarin anticoagulation to reduce bleeding risk is inadequate for secondary prevention of thrombotic events.

Consider the severity of thrombocytopenia

The severity of thrombocytopenia is an important clue to the diagnosis. Platelet counts below $20 \times 10^9/L$ are typical of primary or secondary ITP, especially in the setting of drugs; HIT generally causes a median platelet count nadir of $60 \times 10^9/L$; splenomegaly results in mild thrombocytopenia (platelet counts approximately $100 \times 10^9/L$); and, in sepsis, platelet counts are variable but thrombocytopenia tends to be mild or moderate.

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 after 5-10 days; however, certain drugs such as tirofiban, eptifibatide, or abciximab may cause thrombocytopenia within hours of the first exposure. Rapid-onset HIT can occur after reexposure 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.

Assess for signs of bleeding

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 predominantly are prothrombotic disorders.

Anemia

Anemia is a common problem among hospitalized patient, especially in the ICU. In critically ill patients, it tends to appear early in the ICU course. Approximately 25%-30% of patients in the ICU will have a hemoglobin level $<9 g/dL$ and approximately one-third of critically ill patients will receive a 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 patients, largely based on the realization that transfusion may be associated with an

increase in infectious risk and overall mortality. Moreover, transfused blood may not impart the expected increase in oxygen delivery to tissues due to a number of alterations during storage, such as decreased levels of 2,3-diphosphoglycerate (and thus decreased oxygen unloading capacity), loss of nitric oxide activity, and proinflammatory effects.

A landmark trial investigating the benefit of a liberal or a restrictive transfusion strategy in the ICU was the Transfusion Requirements in Critical Care (TRICC) 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. Several subsequent studies have supported these observations and recent surveys suggest that transfusion practices have changed toward a more restrictive approach.

A recent meta-analysis (45 studies, including 272,596 patients) examined the effect of RBC transfusion on morbidity and mortality in critically ill medical, trauma, and surgical patients. RBC transfusion was an independent predictor of death, infections, multiorgan dysfunction syndrome, and acute respiratory distress syndrome, suggesting that the need for RBC transfusion should be assessed on an individual basis. For example RBC transfusions are warranted as a treatment of bleeding but may not be appropriate as a universal treatment of ICU-associated anemia.

One recent area of controversy is the effect of blood storage time on clinical outcomes of transfusion recipients. In recent meta-analysis of 409,966 patients that included 21 studies, older blood was associated with a significantly increased risk of death (odd ratio, 1.16; 95% confidence interval, 1.07-1.24). Most of the data were derived from observational studies or small randomized trials from select patient populations. Two pilot randomized control trials (RCTs) did not suggest a harmful effect of older blood transfusion, and several large RCTs currently are under way to address this question, including a 24,000-patient pragmatic RCT in unselected hospitalized patients.

Key points

- Life-threatening causes of thrombocytopenia should be considered first in any patient presenting with thrombocytopenia: DITP, heparin-induced thrombocytopenia, TTP, sepsis and DIC, CAPS, and PTP.

Key points (continued)

- The diagnosis of TTP should be considered in any patient with thrombocytopenia and microangiopathic hemolytic anemia.
- DIC is caused by unimpeded thrombin generation. 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.
- Heparin-induced thrombocytopenia is an uncommon cause of thrombocytopenia in patients admitted to the ICU.
- For most patients, RBC transfusions are not required for non-bleeding critically ill patients with a hemoglobin concentration above 7g/L.

Consultation for hematologic complications of solid organ transplantation

Clinical case

You are consulted on a 33-year-old woman with pancytopenia. She underwent renal transplantation 6 weeks ago for end-stage diabetic nephropathy. Over the past week, she has developed a rash and fever. Her laboratory studies demonstrate a white blood cell count of 2,000/ μ l, an absolute neutrophil count of 1,100/ μ l, a hemoglobin of 7.5 g/dL, a platelet count of 37,000/ μ l, and new liver function test abnormalities. She is taking azathioprine, prednisone, and tacrolimus. There have been no clinical or laboratory signs of graft rejection. Blood cultures are negative. The patient was cytomegalovirus (CMV)-seronegative and received a cadaveric allograft from a CMV-positive donor. You recommend CMV nucleic acid testing, peripheral blood lymphocyte chimerism studies, and bone marrow aspirate and biopsy. You also recommend discontinuation of azathioprine and other nonessential medications.

This section offers an approach to the patient with hematologic complications after solid organ transplantation (SOT). One of the most common reasons for hematologic consultation in this setting, as illustrated by the clinical case, is cytopenia. Common causes of posttransplant cytopenia include infection and drugs. Graft-versus-host disease (GVHD) and posttransplant lymphoproliferative disorder (PTLD) involving the bone marrow are rarer etiologies. These conditions, as well as posttransplantation erythrocytosis (PTE) and transfusion support in the posttransplant setting, are discussed in the following sections.

Drug-related complications

Immunosuppressant and antimicrobial drugs are prevalent causes of cytopenia after SOT. 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 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 withdrawal of the offending drug. Although plasma exchange may be attempted in refractory cases, there is little evidence to support its use in this setting.

Alloimmune complications

GVHD is a rare and often fatal complication of SOT. 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 SOTs, patients receiving small bowel 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 SOT patients presents similarly to acute GVHD after hematopoietic stem cell transplantation. Fever, rash, and diarrhea 2–6 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.

Another alloimmune complication of SOT is alloimmune hemolysis of host erythrocytes by passenger lymphocytes (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. 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. Treatment involves

transfusion of compatible RBCs and plasma exchange if hemolysis persists. Passenger lymphocyte syndrome is typically self-limited due to the short survival of donor lymphocytes in the circulation.

Posttransplantation lymphoproliferative disorders

PTLDs make up a group of predominantly B-cell neoplasms that occur in immunosuppressed individuals following SOT. In most cases, B-cell proliferation is induced by Epstein-Barr virus (EBV) infection. PTLD affects ~1% of solid organ recipients. 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 2–8 weeks after initiation of immunosuppression; 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.

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%–70% with rituximab, although RCTs have not been reported. Radiation therapy may be used for treatment of local disease.

Transfusion support

A hematologist may be asked to assist with transfusion management in a patient undergoing SOT. Of all SOTs, transfusion is most commonly required for liver transplantation due to the underlying coagulopathy of liver failure. The typical liver transplantation requires the transfusion of 10–22 allogeneic RBC units, although this amount may be reduced through the use of intraoperative blood salvage. In addition to RBC transfusions, patients undergoing liver transplantation often require large volumes of plasma and platelets. 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 SOT carries the potential risks of infection, human leukocyte antigen (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. More recent 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. Leukocyte reduction can be accomplished during RBC processing or at the bedside with a filter. Plasma exchange, IVIg G, and rituximab have been used in patients with a positive panel of reactive antibodies (PRAs) or major ABO incompatibility to minimize the risk of hyperacute rejection.

Transfusion-associated GVHD is rare among SOT patients, although it is associated with a mortality of 90% or higher. Because of its rarity, irradiation of blood products is not routinely recommended for recipients of solid organ transplants.

Posttransplantation erythrocytosis

PTE is defined as an elevated hematocrit that occurs following renal transplantation and persists for more than 6 months in the absence of leukocytosis, thrombocytosis, or another potential cause of erythrocytosis. PTE affects 8%-15% 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 8-24 months after transplantation. Clinical manifestations include malaise, plethora, headache, and a propensity for both venous and arterial thromboembolism. 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 an SOT 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 8-24 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 which has led to good seizure control. He otherwise is in good health. He reports no episodes of bleeding and there is 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 $80-150 \times 10^9/L$ 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. Chronic thrombocytopenia may suggest the possibility of an inherited process, such as a MYH9-related macrothrombocytopenic disorder, which may be first discovered during pregnancy when women often have their blood tested for the first time. Splenomegaly should be assessed with a physical examination and ultrasound if appropriate.

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. 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, and musculoskeletal abnormalities. An underlying etiology often is not found. Most patients with mild thrombocytopenia (platelet count $100\text{--}150 \times 10^9/\text{L}$) 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, and circulating blasts are consistent with acute leukemia. Dysmorphic red blood cells, hypogranulated neutrophils, or Pelger-Huët cells may suggest underlying myelodysplastic syndrome, which may present with isolated thrombocytopenia in up to 10% of patients.

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 myelodysplastic syndromes 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 should be performed if unexplained symptoms arise or other hematologic abnormalities appear to rule out bone marrow pathology.

Leukocytosis

Patients with unexplained leukocytosis frequently are referred to a hematologist because of the 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.

In addition to examining the peripheral smear, 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 use may indicate a drug-induced leukocytosis. Examination of the skin, lymph nodes, liver, and spleen size is 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. 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.

Persistent polyclonal B-cell lymphocytosis is an unusual disorder of unclear etiology characterized by stable polyclonal expansion of lymphocytes, elevated polyclonal immunoglobulin (Ig) M, and the presence of binucleated lymphocytes on the peripheral smear. It is more common in females, follows a benign course, and rarely progresses to malignancy.

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 complete blood counts (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. Patients with cyclic neutropenia, due to disorders with neutrophil elastase, typically have a 21-day periodicity associated with their neutropenia. 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,

Table 2-4 Hematology consultation for leukocytosis: etiologic considerations according to which cell type is elevated.

Neutrophilia	Monocytosis	Eosinophilia	Lymphocytosis
Eclampsia	Pregnancy	Allergic rhinitis	Mononucleosis syndrome
Thyrotoxicosis	Tuberculosis	Asthma	Epstein-Barr virus
Hypercortisolism	Syphilis	Tissue-invasive parasite	Cytomegalovirus
Crohn's 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)
Granulomatous infections	Corticosteroids	Vasculitides	
Bronchiectasis		Drug reaction	Toxoplasmosis
Occult malignancy		Adrenal insufficiency	Babesiosis
Trauma/burn		Occult malignancy	Drug reaction
Severe stress		Pulmonary syndromes	Reactive large granular lymphocytosis
Panic		Gastrointestinal syndromes	
Asplenia		Hypereosinophilic syndrome	
Cigarette smoking			
Tuberculosis			
Chronic hepatitis			
Hereditary neutrophilia			
Corticosteroids			
β-agonists			
Lithium			

HIV= human immunodeficiency virus.

margination, or sequestration. A list of causes of acquired leukopenias that affect neutrophils, lymphocytes or both is included in Table 2-5.

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. A wide variety of infectious disorders can cause leukopenia, including hepatitis, mononucleosis, HIV, typhoid, and malaria.

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 smear should be evaluated for the presence of blasts, which would indicate acute leukemia, or Pelger-Huët cells, which are seen in myelodysplastic syndrome. Evaluation of the bone marrow with flow cytometry may be helpful to identify a malignant clone. A rheumatologic evaluation, including antinuclear antibody (ANA), and rheumatoid factor may indicate a previously undetected collagen vascular disorder or systemic lupus

Table 2-5 Causes of acquired leukopenia.

Autoimmune
Marrow aplasia
Thymoma
Idiopathic
Hematologic malignancy
Infections
Sepsis
Viral (HIV, CMV, EBV, hepatitis A, B, C, influenza, parvovirus, others)
Bacterial (tuberculosis, tularemia, <i>Brucella</i> , typhoid)
Rickettsial (Rocky Mountain spotted fever, ehrlichiosis)
Fungal (histoplasmosis)
Parasitic (malaria, leishmaniasis)
Drug and chemical induced (corticosteroids, antilymphocyte globulin, carbamazepine, sulfonylureas, others)
Immunodeficiency
Nutritional
Iatrogenic
Autoimmune conditions (systemic lupus erythematosus, rheumatoid arthritis)
Acute respiratory distress syndrome
Increased neutrophil margination (hemodialysis)

CMV = cytomegalovirus; EBV = Epstein-Barr virus; HIV = human immunodeficiency virus.

erythrematosus. Splenomegaly in this setting may suggest Felty's syndrome, although idiopathic causes of splenomegaly may lead to leukopenia. Treatment of leukopenia depends on the specific etiology. Treatment with colony-stimulating factors should not be used without a definitive diagnosis requiring such an intervention or if severe infection occurs in the setting of neutropenia.

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. On physical examination, large size, hard texture, fixed mobility, and the lack of pain are features suggestive of malignancy. Additional investigations for patients with lymphadenopathy might include ANA, monospot, HIV, and a CBC. 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, and immunohistochemistry.

Castleman's 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 (IL-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's disease can be classified pathologically into hyaline vascular variant, plasmacytoid variant, and human herpes virus 8 (HHV8)-positive Castleman's disease. HHV8 encodes a viral IL-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

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
	Other viral infections
Sarcoidosis	Leptospirosis
Langerhans cell histiocytosis	Tularemia
Inflammatory pseudotumor	Miliary tuberculosis
Progressive transformation of germinal centers	Brucellosis
Malignancy (eg, NHL, HD, CLL, metastatic carcinoma)	Lyme disease
	Secondary syphilis
	Toxoplasmosis
	Histoplasmosis
	Systemic lupus erythematosus
	Rheumatoid arthritis
	Still disease
	Rosai-Dorfman disease
	Sarcoidosis
	Langerhans cell histiocytosis
	Phenytoin
	Drug-induced serum sickness
	Castleman's disease
	Kikuchi disease
	Kawasaki disease
	Angioimmunoblastic lymphadenopathy
	Atypical lymphoproliferative process
	Hemophagocytic lymphohistiocytosis
	Malignancy (eg, indolent NHL, CLL)

CLL = chronic lymphocytic leukemia; HD = Hodgkin disease;

HIV = human immunodeficiency virus; NHL = non-Hodgkin lymphoma.

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.

Splenomegaly occurs in patients with cirrhosis when increased portal pressure causes venous engorgement and disruption of the normal splenic architecture. Other causes are splenic vein thrombosis, neoplastic process such as lymphoma, and infiltrative disorders such as Gaucher's disease. Hereditary spherocytosis also may cause splenomegaly because of ongoing hemolysis. 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 because of increased splenic sequestration.

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 infection with encapsulated organisms, patients undergoing splenectomy should be vaccinated for *S. pneumoniae*, *H. influenzae*, and *N. meningitidis* and asplenic individual who develop a fever should be treated promptly with 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. 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.

Key points

- Most patients with stable, mild thrombocytopenia (platelets $100-150 \times 10^9/L$) 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 please refer to Chapters 5-7.

Newborn

Figure 2-3 illustrates the diagnostic approach to anemia in the newborn. At birth, infants are relatively polycythemic and macrocytic, reflecting the hypoxic intrauterine environment and fetal RBC production. Mean hemoglobin and hematocrit on day 1 of life for a term newborn are 19.0 ± 2.2 g/dL and $61\% \pm 7.4\%$, respectively (Table 2-7). Therefore, a hematocrit that would be considered normal during childhood represents anemia in the newborn. Shortly after birth, RBC production decreases as the erythropoietin declines, and by day 7, the reticulocyte count is 0.5%. A physiologic nadir in hemoglobin concentration (10.7 ± 0.9 g/dL) occurs at approximately 7-9 weeks of age and is earlier and more pronounced in premature infants.

Anemia in the newborn requires a careful assessment of the obstetrical and birth history along with review of the family history for jaundice, anemia, splenectomy, or cholecystectomy. Physical examination should focus on findings such as jaundice, vitals signs, and possible sources of internal blood loss. A review of the CBC, red cell indices, reticulocyte count, and peripheral blood smear can narrow the board differential. Additional laboratory testing should be guided by the presence or absence of findings.

Anemia in the newborn can be 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 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 reciprocal twin.

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

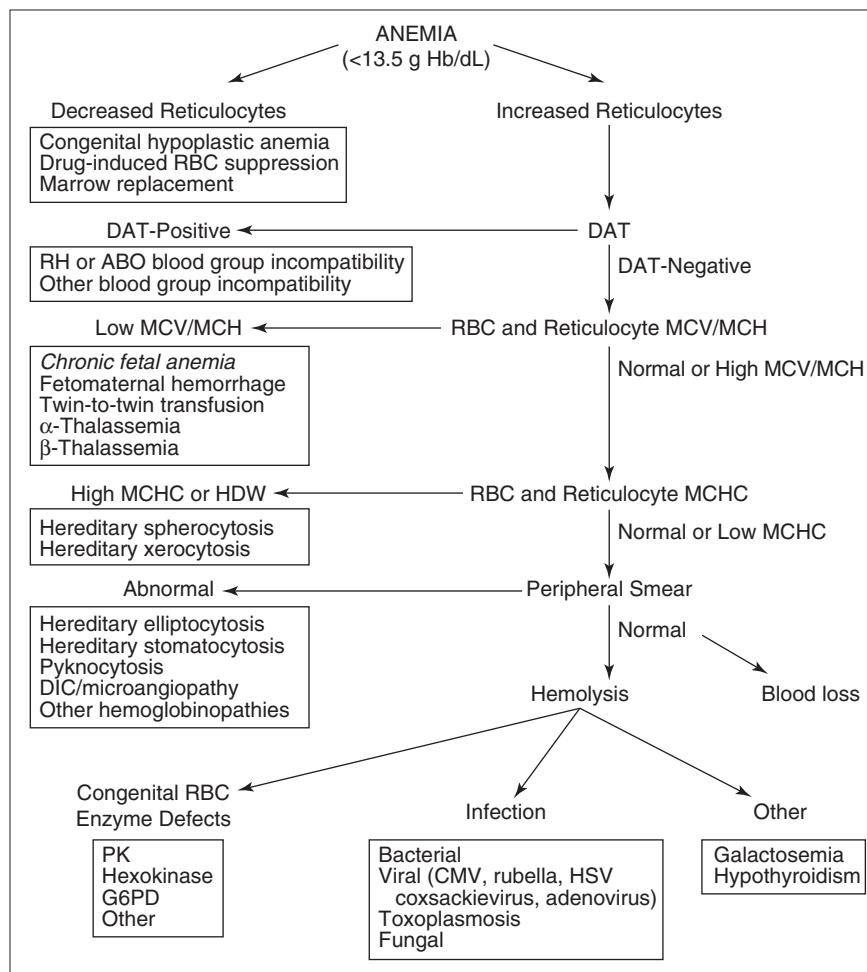


Figure 2-3 Diagnostic approach to anemia in the newborn. From Brugnara C, Platt OS. The neonatal erythrocyte and its disorders. In: Nathan DG, Orkin SH, Ginsburg D, Look AT, eds. *Nathan and Oski's Hematology of Infancy and Childhood*. 6th ed. Philadelphia, PA: WB Saunders; 2003:19-55. CMV = cytomegalovirus; 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.

The infant 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. Immune hemolysis due to ABO incompatibility, currently the most common cause of hemolytic disease of the newborn in counties 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 smear 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%-50% of affected fetuses. Common intrinsic red cell

etiologies include hereditary spherocytosis (HS) and glucose-6-phosphate dehydrogenase (G6PD), 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).

Management of anemia requires evaluation of the possible cause, the 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. In cases of significant 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.

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)

* Adapted from Matoth Y, Zaizov R, Varsano I. Postnatal changes in some red cell parameters. *Acta Paediatr Scand*. 1971;60:317-323.

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

From Reverdieu-Moalic P, Delahousse B, Body G, et al. Evaluation of blood coagulation activators and inhibitors in the healthy human fetus. *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.

Children

Asymptomatic anemia often is discovered at approximately 1 year of life when children undergo a screening hemoglobin. While useful, this does not fully identify the cause of anemia, and follow-up studies, including a CBC and reticulocyte count are recommended. This section provides an overview and details 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 or thalassemia. Iron deficiency is commonly diagnosed around 1-2 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 from cow's milk, it is generally inadequate as a sole source of iron beyond 4-6 months of life. In addition, at 1 year of life, children typically switch to iron-poor cow milk, have inadequate intake of

iron-containing foods, and develop gastrointestinal irritation with poor absorption and occult blood loss secondary to cow milk proteins. A careful diet history usually provides evidence that the child has iron deficiency 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. Direct and repetitive questioning and specific testing may be required to elicit the cause.

Laboratory studies in iron deficiency will show hypochromia, anisocytosis, reticulocytopenia, and microcytosis. Iron stores deplete before changes are seen on the CBC, and the MCV is the last parameter to decline. A low ferritin indicates depletion of iron stores and is confirmatory for iron deficiency; however, a normal ferritin does not exclude the diagnosis as it is an acute-phase reactant. The best confirmatory

test for iron deficiency is response to a therapeutic trial of iron. Within 2 weeks of appropriate iron replacement (4–6 mg/kg/d of elemental iron), reticulocytosis, and improvement of hemoglobin should be observed. The most common reasons children fail iron therapy include noncompliance, improper dosing, and a diagnosis other than iron deficiency. If there is no response to an adequate trial of iron and parents report compliance, this treatment should be stopped and alternative causes, including blood and malabsorption, should be sought.

The most common alternative diagnosis is thalassemia, particularly in children of African American, Mediterranean, or Asian backgrounds. Table 2-8 outlines the gene deletions and corresponding nomenclature for thalassemia. Newborn screening (NBS) will show hemoglobin Bart's in two or three gene α -thalassemia. After hemoglobin switching, 1- or 2-gene α -thalassemia will not be evident on hemoglobin electrophoresis and α -globin gene sequence analysis is necessary to confirm this diagnosis. Children with more severe α -thalassemia will have hemoglobin H. β -thalassemia trait or intermedia may not be detected on NBS; however, β -thalassemia major will have a hemoglobin F only pattern. Repeat electrophoresis at 6–12 months of life after the hemoglobin switching will reveal increased hemoglobin A₂. 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.

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 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 smear 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. Intrinsic causes can be further classified by cause, including: (i) enzyme deficiencies (G6PD), (ii) membrane defects (such as HS), or (iii) hemoglobinopathies (sickle cell disease). 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 count.

Table 2-8 Thalassemia gene deletions and nomenclature.

Gene Deletions	Nomenclature
α-Thalassemia	
$\alpha\alpha/\alpha\alpha$	Normal
$\alpha\alpha/\alpha_-$	Silent Carrier
$\alpha\alpha/_-$	Thalassemia trait
α_-/α_-	Thalassemia trait
$\alpha_-/_-$	Hemoglobin H disease
$_/_-$	Hydrops fetalis
β Thalassemia	
β/β	Normal
β/β^0	β -thalassemia trait
β/β^+	β -thalassemia trait
β^+/β^+	β -thalassemia intermedia
β^0/β^+	β -thalassemia intermedia
β^0/β^0	β -thalassemia major (Cooley's anemia)

β^+ = decreased β -globin gene production; β^0 = absent β -globin gene production.

Primary autoimmune hemolytic anemia (AIHA) can be caused by either IgG (warm-reactive) or IgM (cold-reactive) antibodies and presents with the acute onset of uncompensated anemia. Overall, unlike adults, children with AIHA have a good prognosis with approximately 77% having an acute self-limited condition. Common intrinsic red cell abnormalities include HS and G6PD. HS may present in the neonatal period similar to maternal-fetal incompatibility with a predominance of spherocytes; however, the DAT will be negative and anemia will persist beyond the newborn period. If there is ongoing concern for HS osmotic fragility testing can be performed. G6PD deficiency is an X-linked inherited condition that results in increased red cell sensitivity to oxidative stress. Children can present in the newborn period with jaundice out of proportion to the degree of anemia. Testing for G6PD during a hemolytic crisis may result in false-negative results as G6PD levels decline with RBC age. Therefore increased reticulocyte count and destruction of aged RBCs may cause levels to be falsely elevated.

TEC is a normocytic anemia with reticulocytopenia resulting from brief disruption of normal erythropoiesis. 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 smear. An important and often unrecognized cause of normocytic anemia is anemia of inflammation (previously anemia of chronic disease). Inflammation leads to an increase in hepcidin, which blocks iron utilization, resulting in iron-restricted anemia. Patients will have a mild normocytic or microcytic anemia with

reticulocytopenia. Anemia of inflammation can be differentiated from iron deficiency because the ferritin is elevated and the total iron-binding capacity (TIBC) is reduced.

Macrocytosis in childhood should always cause concern and a bone marrow evaluation should be undertaken looking for causes of a 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 five other ribosomal protein genes now have been identified. Treatment modalities include corticosteroids, chronic transfusions, and bone marrow transplant.

Neutropenia

Newborn

Neutropenia 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 (PIH). In both cases the neutropenia is transient and resolves with resolution of the underlying illness or in the case of PIH within 3-5 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 outside of the neonatal period 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 antineutrophil antibodies can be detected; however, due to the poor sensitivity of antibody testing, a negative result does not exclude the diagnosis. Management is directed at treating infections with antibiotics, and G-CSF should be reserved for severe or recurrent infections associated with a low absolute neutrophil count. Prognosis is excellent with spontaneous recovery occurring in almost all patients.

Inherited causes of neutropenia represent a rare group of disorders, including severe congenital neutropenia (SCN), Shwachman-Diamond syndrome (SDS), 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. SDS 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 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 1-2 times a week for 6-8 weeks can help confirm the diagnosis. In all cases, treatment with G-CSF is standard of care. Less clear is the role of bone marrow transplant for those conditions that are considered premalignant.

Thrombocytopenia

Newborn

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, and therefore, a critical first question is whether the child is well or ill. For example, thrombocytopenia is seen in up to half of newborns admitted to a neonatal ICU and may be severe. Causes of secondary thrombocytopenia in this setting include perinatal asphyxia, respiratory distress, sepsis, polycythemia, necrotizing enterocolitis, and intrauterine viral infections.

In an otherwise-well child, ITP should be investigated. Knowledge of maternal medical history and platelet count is critical as management varies depending on suspicion for alloimmune versus autoimmune thrombocytopenia. Autoimmune thrombocytopenia, either primary or secondary,

presents early in infancy due to transplacental passage of maternal platelet-reactive IgG, 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 cause 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 platelet count is normal. NAIT results from the transplacental passage of maternal antibody, which is 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 associated with NAIT is associated with a high risk of intracranial hemorrhage (ICH; 10%-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 ICH 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. IVIg (1.0 g/kg/d for 1-3 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 2-4 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.

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. ITP in children can be either primary or secondary and 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 smear. A bone marrow examination is not considered necessary for the diagnosis of ITP.

Treatment of the child with ITP remains controversial. Recently 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 have spontaneous recovery of their platelet counts, 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 U.S. Food and Drug Administration. Short courses of corticosteroids are effective and are 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 included intermittent use of medications, splenectomy, or newer modalities such as rituximab, high-dose dexamethasone, and thrombopoietin (TPO) receptor agonists. Splenectomy usually is avoided in children who are younger than 5 years old due to the lifelong risk of sepsis in splenectomized patients and in children with ITP for <1 year due to the potential for spontaneous remission. The benefit is a high rate of durable remission, which occurs in approximately 75% of patients. 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%-30%; however, remission duration is generally shorter than with splenectomy. The new TPO receptor agonists are approved for the treatment of ITP in adults, but their role in pediatrics is not yet established.

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 (ALPS) results

from impaired *fas* ligand–mediated apoptosis. Patients experience recurrent lymphadenopathy, organomegaly, and immune cytopenias. Kasabach-Merritt syndrome is characterized by thrombocytopenia and giant hemangiomas during infancy. Patients can develop a severe life-threatening consumptive coagulopathy and many treatment modalities have been described, including corticosteroids and vincristine.

Causes of decreased platelet production include aplastic anemia, myelodysplastic syndrome, bone marrow infiltration, and congenital thrombocytopenias. The congenital thrombocytopenias represent a diverse group of disorders (see Chapter 10). 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 smear provide important diagnostic clues. Microthrombocytopenia in males should raise the possibility of Wiskott-Aldrich syndrome (WAS) or X-linked thrombocytopenia (XLT), caused by a mutation in the WAS gene. WAS, unlike XLT, 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 of management for patients with congenital thrombocytopenia, accurate diagnosis is important, as some conditions are associated with transformation to leukemia and may benefit from bone marrow transplant.

Coagulopathy

Newborn

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%–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 the 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 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 heelstick blood draws, or more bleeding or bruising than expected from a difficult delivery, should raise the possibility of an inherited bleeding disorder. Screening can be undertaken with a PT and aPTT, with specific factor levels based on results.

The most common inherited causes of an isolated aPTT in an otherwise-healthy infant are factor VIII and factor IX deficiency. 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 associated with an elevated aPTT, 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, fresh frozen plasma (FFP) will provide adequate hemostatic coverage.

Elevation of both the PT and aPTT should prompt investigation for vitamin K deficiency. Although all infants born in the hospital receive supplemental vitamin K, home deliveries and parental desire to avoid medical interventions has increased the incidence in breastfed 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 1–2 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.

If there is a high suspicion for a coagulopathy, 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 clot solubility

test can be performed to screen infants with concerning history.

Children

The diagnostic work-up for a child with a suspected coagulopathy begins with a thorough history and screening for PT and aPTT. Specific considerations for additional testing depend on concerns identified on history and screening laboratory examination.

If an abnormality is identified, laboratory error or heparin contamination should be considered and eliminated as a possible cause. Otherwise, a common cause of isolated aPTT elevation in a healthy child with no bleeding history or symptoms is a lupus anticoagulant. In this setting, transient antibodies are interfering with the phospholipids that are required to perform the aPTT. The condition usually is self-limited and does not represent a bleeding disorder in the child. Confirmatory testing for a lupus anticoagulant can be undertaken if it is felt necessary or if there is a question of the diagnosis. Patients with a concerning history should be elevated for a factor deficiency. Family history may provide information to guide testing, with factor VIII and IX deficiency having an X-linked inheritance. 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. 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.

An isolated prolonged PT represents a deficiency of factor VII. Inherited factor VII deficiency is a rare autosomal bleeding disorder with variable presentation. Beyond congenital factor VII deficiency, consideration should be given to acquired causes 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 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 factors VIII, V, and II often can provide information to distinguish these etiologies if it is not clinically apparent. In DIC, all three will be decreased; in liver disease, factor VIII will remain normal; and in vitamin K deficiency, only factor II will be decreased.

In all cases, treatment should be aimed at reducing hemorrhage and at correcting the 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 specific factor is not available, the deficiency is not known, or multiple factors are involved then FFP can be given.

Thrombosis

Newborn

Similar to pregnancy, the balance between hemostasis and fibrinolysis is shifted toward thrombosis in the newborn. Although antithrombin III levels in neonates are mildly lower than in adults and the vitamin K-dependent anticoagulants, proteins C and S, are 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 venous sinus thrombosis. Clinically, it may be difficult to determine whether the thrombotic event occurred pre- or postnatally.

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 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. Genetic testing can be performed to confirm a congenital cause but should not delay treatment. Immediate treatment should be initiated 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 S replacement should be administered for 6-8 weeks, until all lesions have healed and a therapeutic international normalized ratio has been achieved.

Beyond protein C and S deficiency, anticoagulation therapy 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. Dosing of tPA may be somewhat higher in newborns compared with 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, but 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 0.5-1 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 are less common. Testing for thrombophilia in children with thrombosis or family history remains controversial and 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 child without other causes for spontaneous thrombosis. MTHFR and homocysteine testing has been largely abandoned due to unclear significance. 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 8).

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 should be 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 to develop cytopenias secondary to poor bone marrow reserve in the setting of stress.
- 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
Margaret V. Ragni and Sarah H. O'Brien

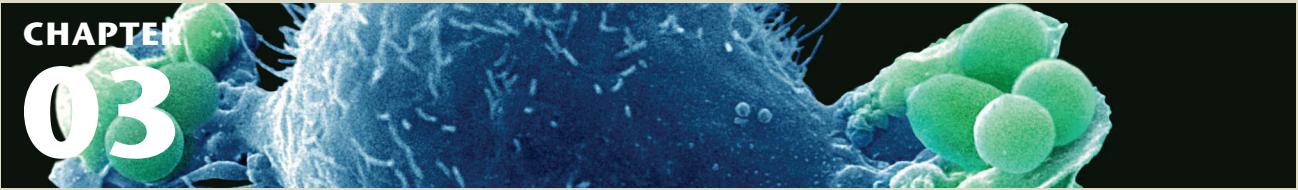
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CHAPTER
03



Consultative hematology II: women's health issues

Margaret V. Ragni and Sarah H. O'Brien

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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 requires 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). Whether the patient is an adolescent, pregnant, or a perioperative or critically ill female patient, 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 outcomes. Furthermore, the plan of care should be formulated with the

multidisciplinary team, when available, utilizing evidence-based guidelines from expert panels of the National Heart, Lung, Blood Institute (NHLBI), National Hemophilia Foundation (NHF), American College of Obstetrics and Gynecology (ACOG), American College of Chest Physicians (ACCP), and World Health Organization (WHO) and 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 in 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, and formulary committees, or developing clinical practice guidelines, establishing policies and procedures for transfusion services, monitoring quality of care and service efficiency, developing practice guidelines, 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 providing patient consultation are just as critical as those required when working on 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 will ensure the highest quality of patient care and optimal patient outcomes.

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Table 3-1 Principles of management of women's hematologic health issues.

Principle	Comment
Women with blood disorders are at increased risk for complications of pregnancy and the menopausal period, including bleeding and clotting in the antepartum, peripartum, and postpartum periods.	Establishing diagnosis of a blood disorder as early as possible, preferably preconception, is essential in optimal management of women with blood disorders. A prediagnosis should be based on clinical and laboratory assessment. A personal and family history of bleeding or clotting, drug history (including hormonal agents), previous pregnancy loss, or pregnancy complications should be determined.
Perform laboratory testing for presence of a blood disorder (eg, a hemostatic disorder), as indicated by personal and family history.	For a woman with suspected blood disorder, a bleeding or thrombotic complication of pregnancy, or disorder predating pregnancy, a thorough history, clinical assessment should be obtained, and laboratory testing performed as indicated.
Involve a multidisciplinary team in the management of women with blood disorders, and develop and discuss the plan with the patient.	General management requires a multidisciplinary team, including hematologist, internist, obstetrician-gynecologist, family practitioner, pediatrician, anesthesiologist, and surgeon, and the availability of specialized laboratory, pharmacy, and blood bank support.
Develop a management plan with the multidisciplinary team with patient-specific recommendations.	Patient management should be coordinated with the multidisciplinary team. Specific recommendations should be communicated regarding laboratory monitoring, treatment guidelines, including factor, blood products, or antithrombotic agents.
Communicate the management plan for delivery with the multidisciplinary team and discuss the plan with the patient.	Ensure all management plans are communicated in a timely fashion with all members of the multidisciplinary team, and also with the patient. Resolve questions and readjust to optimize compliance.
Anticipate postoperative, postprocedure therapy, including duration and where and by whom it will be given.	Initiate postprocedure, postdelivery, postoperative planning before the event, and include plans for the dose, duration, and location of postprocedure care, involving the patient in the decision making.
Offer educational information and guidance.	Provide relevant evidence-based literature and guidelines to colleagues and to patients, as requested.
Provide information to the multidisciplinary team and patient.	Continue involvement and progress notes as indicated, particularly in vulnerable periods such as postpartum. Provide for outpatient monitoring, treatment, and follow-up, with ongoing communication with the multidisciplinary team and patient.

Adapted from Nichols WL, Hultin WB, James AH, Manco-Johnson MJ, Montgomery RR, Ortel TL, Rick ME, Sadler JE, Weinstein M, Yawn BP. von Willebrand disease (vWD): evidence-based diagnosis and management guidelines. The National Heart Lung Blood Institute (NHLBI) Expert Panel Report (USA). *Haemophilia*. 2008;14:171-232; and MASAC Recommendations regarding girls and women with inherited bleeding disorders. Medical and Scientific Advisory Committee, *National Hemophelia Foundation*. 2010, #197; and Ragni M. Management of pregnancy in women with von Willebrand disease. *Treatment Strategies-Hematology*. 2012;2:88-92.

Hematologic health issues in pregnancy

Anemia in pregnancy

During normal pregnancy, the plasma volume expands by 40%-60%, whereas the red blood cell mass expands by 20%-50%. Thus, a physiologic anemia develops, leading to a normal hematocrit value of 30%-32%. Hemoglobin levels <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 to 34% in the third trimester. The latter is a major indicator of reproductive health. A goal of Healthy People 2010 was to reduce third trimester anemia to 20% or less.

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 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 deficiency. In the absence of iron supplementation, hemoglobin falls to 10.5 gm/dL at 27-30 weeks gestation; with iron supplementation, the nadir is less severe, 11.5 gm/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 15-30 mg daily of supplemental elemental iron, although studies examining the efficacy of iron supplementation during pregnancy have not shown a clear benefit to pregnancy outcomes. For patients who do not tolerate oral iron, parenteral iron may be used. Iron sucrose is categorized as pregnancy class B (presumed safe based on animal models) and is preferred over iron dextran, which is considered pregnancy class C (safety uncertain). Recent studies have demonstrated that for patients who do not respond well to parenteral iron, the addition of recombinant erythropoietin may add benefit. In a study of 40 patients with gestational iron deficiency anemia who had unsatisfactory responses to oral iron, 20 patients were randomized to receive recombinant erythropoietin (rEPO) and parenteral iron sucrose (group 1) and 20 were randomized to receive iron sucrose alone (group 2). Patients in group 1 displayed higher reticulocyte counts on day 4, greater increases in hemoglobin from day 11, and a shorter duration of therapy to reach the target hemoglobin of 11 g/dL. No abnormalities in fetal hemoglobin levels were observed, consistent with the belief that rEPO does not cross the placenta. Thus, 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, particularly given the increased risk of thrombosis during 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.

Management: For pregnant women, daily 15-30 mg elemental iron is recommended. For those not able to tolerate oral iron, parenteral iron sucrose is preferred.

Megaloblastic anemia

The majority of macrocytic anemias during pregnancy are due to folate deficiency, whereas vitamin B12 deficiency is rare. Multivitamin and folic acid supplementation reduce placental abruption and recurrent pregnancy loss. Folate requirements increase from 50 mg daily in the nonpregnant female to at least 150 mg daily during pregnancy, and the Centers for Disease Control and Prevention recommend

supplementation with 400 mg daily of folate to prevent neural tube defects. Folate deficiency is most precisely diagnosed by measuring plasma levels of homocysteine and methylmalonic acid.

Management: For pregnant women, daily folic acid 400 mcg is recommended.

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 idiopathic thrombocytopenia (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. Aplastic anemia may lead to maternal death in up to 50% of cases, usually caused by hemorrhage or infection, and in utero fetal complications may occur in one-third. 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/ μ l, growth factors (eg, granulocyte colony-stimulating factor [G-CSF]), and, in select cases, cyclosporine. 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.

Management: For pregnant women with aplastic anemia, transfusions to maintain a platelet count of >20,000/ μ l, and growth factors (eg, G-CSF), as needed, are recommended.

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 is 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%-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, low platelets (HELLP), are challenging to diagnose, given the wide overlap in clinical presentation, and are difficult to treat, given

disparate treatments. These are discussed in the section "Thrombocytopenia in Pregnancy." Recommendations are provided for each disorder.

Thrombocytopenia in pregnancy

Thrombocytopenia affects 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.

Gestational thrombocytopenia

Isolated thrombocytopenia most commonly results from "gestational" or "incidental" thrombocytopenia of pregnancy. Gestational thrombocytopenia usually develops during the second or third trimester in otherwise-healthy pregnant women. Thrombocytopenia is usually mild, and by definition, the platelet count does not decrease $<70,000/\mu\text{L}$. There is no diagnostic test for gestational thrombocytopenia, so it is a diagnosis of exclusion. This disorder may represent an extreme example of the typical 10% decrease in platelet count that occurs during normal pregnancy. Gestational thrombocytopenia does not affect pregnancy outcome and does not result

in thrombocytopenia in the offspring of affected women; thus, no specific treatment is required. It is usually self-limited and resolves postpartum. 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.

Management: No treatment is recommended, as the disorder generally resolves postpartum.

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, as antibody testing lacks specificity. A prior history of thrombocytopenia or autoimmune disease preceding pregnancy or during previous pregnancies is useful in making a diagnosis of ITP. Patients with ITP generally present with more severe thrombocytopenia than those with gestational thrombocytopenia, but the two disorders may be indistinguishable when ITP is mild. Indications for treatment of ITP in pregnancy in the first two trimesters include: (i) when the patient is symptomatic, (ii) when platelets fall $<20,000-30,000/\mu\text{L}$, or (iii) to increase platelet count to a level considered safe for procedures. Although the lowest platelet count safe for spinal or epidural anesthesia is controversial, most obstetric anesthetists recommend a platelet count of $75,000/\mu\text{L}$, and

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-severe	–	–	–	–	–	–	Anytime, common in first trimester
Gestational	Mild	–	–	–	–	–	–	Second and third trimester
Preeclampsia	Mild-moderate	Mild	Absent-mild	Moderate-severe	–	Proteinuria	Seizures in eclampsia	Late second to third trimester
HELLP	Moderate-severe	Moderate-severe	May be present (mild)	Absent-severe	Moderate-severe	Absent-moderate	Absent-moderate	Late second to third trimester
HUS	Moderate-severe	Moderate-severe	Absent	Absent-mild	Absent	Moderate-severe	Absent-mild	Postpartum
TTP	Severe	Moderate-severe	Absent	Absent-severe	Absent	Absent-moderate	Absent-severe	Second to third trimester
AFLP	Mild-moderate	Mild	Severe	Absent-severe	Severe	Absent-mild	Absent-severe	Third trimester

Proteinuria measured as total protein excreted in 24 hours is rated as follows: normal $<150 \text{ mg}/24 \text{ h}$; mild is $150-300 \text{ mg}/24 \text{ h}$; moderate is 300 mg to $1 \text{ gm}/24 \text{ h}$; severe is $\geq 1 \text{ gm}/24 \text{ h}$.

AFLP = acute fatty liver of pregnancy; CNS = central nervous system; HELLP = hemolysis, elevated liver function tests, low platelets; HUS = hemolytic uremic syndrome; ITP = idiopathic thrombocytopenic purpura; MAHA = microangiopathic hemolytic anemia; TTP = thrombotic thrombocytopenic purpura.

most hematologists recommend for at least 50,000/ μ L for cesarean 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 given initially at low dose, 10–20 mg/day, with adjustment to the minimum dose providing a hemostatically effective platelet count. Although short-term, low-dose prednisone is considered effective safe in the mother, it may exacerbate hypertension, hyperglycemia, osteoporosis, weight gain, and psychosis. Intravenous anti-D has been used successfully to treat ITP in Rh(D)-positive women, although 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 in pregnant individuals, although B-cell lymphocytopenia has been reported in the offspring of individuals treated with this agent, which is considered pregnancy class C. The thrombopoietic agents romiplostim and eltrombopag also are considered pregnancy class C, and 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.

Management: For pregnant women, 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.

Up to 10% of the offspring of patients with ITP also will be thrombocytopenic, and 5% will have platelet counts <20,000/ μ 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 cesarean delivery 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 the 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 explains the abandonment of PUBS in recent years.

Management: All offspring of patients with ITP should be monitored closely for the development of ITP within the first 4–7 days after delivery, and all thrombocytopenic neonates should undergo cranial ultrasound. For severely affected offspring, IVIg is recommended.

Preeclampsia, eclampsia

Thrombocytopenia also may occur in patients with preeclampsia. Preeclampsia affects 6% of all first pregnancies and usually develops in the third trimester; its diagnostic features include hypertension and proteinuria (>300 mg/24 h). Preeclampsia occurs most commonly in primagravidas <20 and >30 years of age. Eclampsia, defined by the presence of grand mal seizures accompanying preeclampsia, complicates <1% of preeclamptic pregnancies. Some studies suggest that preeclampsia may be more common in patients with thrombophilia, particularly those with antiphospholipid antibody syndrome (APLAs). 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 low-dose aspirin, in women with preeclampsia. In a recent Cochrane review of 43 randomized trials including over 32,000 patients, antiplatelet agents significantly reduced preeclampsia in both those women at low and high risk for preeclampsia, if started before 20 weeks gestation. Although the use of anti-thrombotic therapy, primarily low-molecular weight heparin (LMWH), has been suggested by some for management of high-risk preeclampsia (those with past preeclampsia, a body mass index [BMI] >35 kg/m², preexisting diabetes, twin pregnancy, family history of preeclampsia, chronic hypertension, renal disease, autoimmune disease, or an underlying angiotensin-converting enzyme insertion or deletion polymorphism), its use remains equivocal. In an observational study of women at high risk for preeclampsia, the addition of LMWH to aspirin resulted in no greater risk reduction than with aspirin alone, whereas a more recent study of high-risk women, found dalteparin resulted in lower risk for severe preeclampsia, low birth rate, or placental abruption than no dalteparin. Caution should be taken in interpreting these results as it is not known whether findings in women with angiotensin-converting enzyme (ACE) mutations apply to all women with preeclampsia. Finally, disseminated intravascular coagulation (DIC) also may accompany severe preeclampsia and be initiated by such processes as retained fetal products, placental abruption, or amniotic fluid embolism. Nevertheless, DIC in this setting

can be severe, abrupt, and fatal if not managed appropriately. Recent studies have indicated that LMWH may reduce the rate of recurrent hypertensive disorders of pregnancy before 34 weeks gestation.

Management: For pregnant women considered to be at risk for preeclampsia, and for those with a previous history of preeclampsia, low-dose aspirin (but not thromboprophylaxis) is recommended throughout pregnancy, starting with the second trimester.

Hemolysis, elevated liver function, low platelets

The HELLP (hemolysis, elevated liver function tests, low platelets) syndrome affects 0.1%-0.89% of all live births and is associated with a maternal mortality of 0%-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%-80% of patients with HELLP coexist with preeclampsia, which by definition has hypertension and 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 32-34 weeks of gestation at the time of presentation, prompt delivery is undertaken (Table 3-3). If cesarean delivery is required, red cells, platelets, formalin-fixed paraffin (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 essential. 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 3-4 days in the majority of patients. HELLP, in particular, occasionally may worsen or even develop postpartum. Pre- 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 5-7 days after delivery.

Management: 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.

Thrombotic thrombocytopenic purpura

The incidence of TTP is increased during pregnancy. It is estimated that 10%-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 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 superadhesive 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), as do infants of affected mothers. A major dilemma in the management of TTP is the difficulty in diagnosis, as overlap with other pregnancy-specific disorders, such as HELLP may delay diagnosis and lead to increased morbidity and mortality. Plasmapheresis is the preferred therapy with a response rate reaching 75%. Corticosteroids also have been used, with responses in 26%, but antiplatelet agents do not appear to be helpful. TTP relapse and fetal loss in subsequent pregnancies is common.

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 second 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, is recommended.	
Thrombotic thrombocytopenic purpura (TTP) For women with TTP, delivery of the fetus and supportive care of the mother, which may include plasma exchange, is recommended.	
Hemolytic uremic syndrome (HUS) For women with HUS, delivery of the fetus and supportive care of the mother, which may include plasma exchange, is recommended.	
Acute fatty liver of pregnancy (AFLP) For women with AFLP, delivery of the fetus and supportive management of the mother is recommended.	Coagulation support is recommended for liver dysfunction and disseminated intravascular coagulation if present.

Adapted from Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. Venous thromboembolism, thrombophilia, antithrombotic therapy, and pregnancy. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (9th ed.). *Chest*. 2012;141(2 suppl):e691S–e736S; Woudstra DM, Chandra S, Hofmeyr GJ, et al. Corticosteroids for HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome in pregnancy. *Cochrane Database Syst Rev*. 2010; 9:CD008148; George JN. How I treat patients with thrombotic thrombocytopenic purpura. *Blood*. 2010;116:4090–4099; Sanchez-Corral P, Melgosa M. Advances in understanding the etiology of atypical hemolytic uremic syndrome. *Br J Hematol*. 2010;150:529–542; Fesenmeir MF, Coppage KJH, Lambers DS, Barton JR, Sibai BM. Acute fatty liver of pregnancy in 3 tertiary care centers. *Am J Obstet Gynecol*. 2005;192:1416–1419.

Management: For obstetric TTP, delivery of the fetus and supportive management of the mother is recommended, which may include plasma exchange or corticosteroids.

Management: For obstetric HUS, delivery of the fetus and supportive management of the mother is recommended, which may include plasma exchange or corticosteroids.

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 3–4 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 3-3). Treatment is similar to that for obstetric TTP. There is no consensus on the risk of developing recurrent TTP or HUS in subsequent pregnancies, although the nonprospectively collected literature suggests that this risk may be relatively high (10%–20%), a recent registry report did not confirm this.

Acute fatty liver of pregnancy

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 and acquired antithrombin deficiency. 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.

Management: For AFLP, delivery of the fetus and supportive management of the mother, which may include coagulation support for liver dysfunction or DIC, if present, is recommended.

Bleeding disorders in pregnancy

Postpartum hemorrhage (PPH) is a major cause of morbidity and mortality in childbirth. Women with an underlying bleeding disorder are at greater risk for PPH. It is estimated that between 20% and 30% have excess bleeding following delivery, about 1.5-fold greater than women without a bleeding disorder. Bleeding disorders and their management in pregnancy, including preferred agents, target levels, and dosing is found in Table 3-4. 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%-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 are uterine atony, retained placenta, and genital tract trauma. Women with inherited bleeding disorders have these same risk factors, as well as the additional risk factor of their coagulation defect. In the general population, most PPHs are primary, with <1% occurring more than 24 hours after delivery. In women with bleeding disorders, delayed (or secondary) PPH, is much more common and has been reported in 20%-25% of women with von Willebrand disease (vWD), 2%-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 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 3-3). 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%-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 21-27 days. A recent case-control study revealed that women with inherited bleeding disorders have

significantly longer postpartum bleeding when compared with controls, even when they receive appropriate hemostatic treatment. In vWD, the pregnancy-induced increase in coagulation factors starts to decline 3 days after delivery, and levels return to prepregnancy levels within 7-21 days of delivery. Therefore, close postpartum monitoring of women with bleeding disorders is recommended for, at minimum, 3 weeks.

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 results from a partial, quantitative deficiency of vWF and accounts for 60% of all vWD cases. Type 2 vWD, accounting for 35% of the disease, consists of four subtypes: type 2A is caused by a qualitative defect in vWF in which high-molecular weight vWF (HMW 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 factor VIII, which may be confused for 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, generally beginning in the early second trimester and peaking 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 or within 6-8 weeks of delivery, lactation, or hormonal contraceptive use. 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 34-36 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

Table 3-4 Management of bleeding disorders in pregnancy.

Bleeding disorder	Factor at delivery	Dosing at delivery	Target at delivery
von Willebrand disease			
Type 1 vWD	vWF concentrate	60-80 IU/kg, then 40-60 IU/kg q 8-12 h, then daily 3-5 days	vWF:RCo >50 IU/dL
Type 2 vWD (2A, 2B, 2M, 2N)	vWF concentrate	60-80 IU/kg, then 40-60 IU/kg q 8-12 h, then daily 3-5 days	vWF:RCo >50 IU/dL
Type 3 vWD	vWF concentrate	60-80 IU/kg, then 40-60 IU/kg q 8-12 h, then daily 3-5 days	vWF:RCo >50 IU/dL
Hemophilia carrier			
Hemophilia A carrier	FVIII concentrate	25-50 IU IV push, for up to 3-4 days	FVIII:C >50 IU/dL
Hemophilia B carrier	FIX concentrate	75-100 IU IV, for up to 3-4 days	FIX:C >50 IU/dL
Rare bleeding disorder			
FI deficiency	Cryoprecipitate, FI concentrate	1-2 unit/10 kg, then every 3-7 days 70 mg/kg, then every 3-7 day	FI >100-150 mg/dL
FII deficiency	FFP, FEIBA	FFP 15-20 ml/kg or FEIBA 20-30 IU/kg	FII >25 IU/dL
FV deficiency	FEIBA	FFP 20 ml/kg; FEIBA 75-100 IU/kg	FV >15 IU/dL
FVII deficiency	FVIIa concentrate	FVIIa 15-20 µg/kg every 3 hours, then taper	FVII >40-60 IU/dL
FX deficiency	FFP, FEIBA	FFP 20 ml/kg; FEIBA 20-30 IU/kg every 48 h	FX >10-20 IU/dL
FXI deficiency	FFP	FFP 15-20 ml/kg; and/or antifibrinolytics	FXI >10-20 IU/dL
FXII deficiency	NA (no bleeding)	NA	NA
FXIII deficiency	Cryoprecipitate, FXIII concentrate	1-2 unit/10 kg every 3-4 weeks 50-75 U/kg, then 10-20 U/kg every 5-6 weeks	FXIII >30 IU/dL

Adapted from Nichols WL, Hultin WB, James AH, Manco-Johnson MJ, Montgomery RR, Ortel TL, Rick ME, Sadler JE, Weinstein M, Yawn BP. von Willebrand disease (vWD): evidence-based diagnosis and management guidelines. The National Heart Lung Blood Institute (NHLBI) Expert Panel Report (USA). *Haemophilia*. 2008;14:171-232; and Kadir R, Chi C, Bolton-Maggs P. Pregnancy and bleeding disorders. *Haemophilia*. 2009;15:990-1005.

vWD = von Willebrand disease; vWF = von Willebrand factor; vWF:RCo = ristocetin cofactor activity; FVIII:C = factor VIII activity; FIX:C = factor IX activity.

in patients with type 2 vWD, functional levels may not be significantly enhanced due 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 and delivery; however, in women with a severe bleeding history or levels below 50 IU/dL at the time of testing, vWF levels should be maintained at delivery at or above 50 IU/dL. This recommendation is based on case series and expert opinion, as there are no randomized trials regarding safe vWF levels for regional anesthesia. If epidural anesthesia is used, 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 2-3 weeks, and it may be unpredictable and occasionally precipitous; thus, the period of 3-6 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]) is contraindicated with neuraxial anesthesia as the latter requires fluid bolusing, which is contraindicated with DDAVP. Thus, for patients with type 1 vWD, including those who are allergic or unresponsive to DDAVP, and for those patients with type 2 and type 3 vWD, vWF concentrate is recommended and continued for up to 3-5 days postpartum, as required by disease severity (Table 3-4).

Management: For pregnant women with type 1 vWD and vWF <50 IU/dL in the eighth month of pregnancy and past severe bleeding history, or unresponsive or allergic to DDAVP, and for those with type 2 or 3 vWD, vWF concentrate is recommended for neuraxial anesthesia and for up to 3-5 days postpartum. Monitoring for postpartum bleeding is recommended for at least 3 weeks and preferably 6 weeks postpartum.

Hemophilia carriers

Postpartum bleeding may occur in 10% hemophilia carriers and may lead to significant blood loss and anemia, in some cases requiring transfusion. 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 15% may be considered to be mild hemophilia. In a small case series of 16 deliveries in five hemophilia B carriers, postpartum bleeding was reduced significantly in those women receiving at least 4 days of factor IX replacement, as compared with fewer than 4 days of replacement.

Management: For hemophilia A (or B) carriers with FVIII (or FIX) levels <50 IU/dL or severe past bleeding history, recombinant factor VIII (or IX) concentrate is recommended at the time of neuraxial anesthesia and continued for up to 3-4 days postpartum. In women with hemophilia in whom an affected infant is anticipated, because of the potential risk of central nervous system bleeding, caesarean delivery should be offered. Vacuum and forceps should be avoided because of the risk of cephalohematoma and intracranial hemorrhage. 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.

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 no bleeding to severe bleeding, 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 during pregnancy, 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 >0.6 g/L, FII >25 IU/dL, FV >15 IU/dL, FX >10-20 IU/dL, FXI >10-20 IU/dL, and FXIII >20-30 IU/dL are recommended, respectively, for each deficiency state, at the time of delivery. FVII and FXI may require no replacement therapy (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%-2% fetal loss. As noted, a team approach

with a coordinated management plan optimizes outcomes for affected women.

Management: For rare bleeding factor deficiency states, FFP or factor concentrate to bring factor levels to hemostatic levels (Table 3-4) at the time of neuraxial anesthesia and for 3 days postpartum is recommended.

Thromboembolism and thrombophilia in pregnancy

Pregnant women are at increased risk for the development of thromboembolism. Venous thromboembolism (VTE) affects 1-2 of 1,000 pregnancies. The relative risk of VTE during pregnancy and the postpartum period is 4.2, with an overall incidence of 199.7/100,000 in a primarily white population. Furthermore, VTE is the leading cause of maternal death, accounting for 1.2 to 4.7 deaths per 100,000 pregnancies. VTE is symptomatic in 5-12 per 10,000 pregnancies and in 3-7 per 10,000 deliveries, for a seven- to tenfold increased risk for VTE in the antepartum period and a 15 to 35-fold increased risk in the postpartum period. Approximately 80% of VTE events are deep venous thrombi (DVT), whereas 20% are pulmonary emboli. Moreover, the rate of VTE is approximately fivefold higher in the postpartum period than during pregnancy, with one-third of pregnancy-associated DVT and one-half of pulmonary emboli occurring after delivery. The risk of arterial thromboembolism is also increased approximately three- to fourfold in pregnant women, reflecting the hypercoagulable state associated with pregnancy.

Pregnancy-associated venous thromboembolism

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 predominance of VTE may reflect compression of the left iliac vein between the right iliac artery and lumbar vertebrae. A diagnosis of VTE 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. Other options are associated with radiation risk to the fetus, although low. On the basis of the American Thoracic Society (ATS) and the Society of Thoracic Radiology (STR) guidelines for diagnosis of VTE, also adopted by the ACCP and ACOG, chest radiography (CXR) is recommended as the first-line radiation-associated procedure for diagnosis of

pulmonary embolism (PE); use of lung scintigraphy is preferred if the CXR is normal; and computed tomographic pulmonary angiography is preferred if the ventilation-perfusion scan is negative. Multidetector helical computed tomography scanning has a low fetal radiation exposure, about 0.013 mSv, but is lower than radiation exposure to the fetus by pulmonary angiography, performed with shielding by the brachial route, <0.5 mSv, or femoral route, 2.21-3.74 mSv (1 mSv = 0.1 rad of radiation). As D-dimer is elevated in pregnancy, it should not be used to exclude PE in pregnancy. Blood hypercoagulability is the major cause of thrombosis in pregnant women. 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-I), all of which return to normal beginning 2-3 weeks postpartum. In parallel, a substantial decrease in the levels of free protein S occurs because of increased levels of C4b binding protein. An increase in activated protein C resistance in the absence of factor V Leiden (FVL), unexplained by the decrease in free proteins S, also is observed in many pregnant patients, particularly in the third trimester. The anticoagulant of choice in the management of VTE arising during pregnancy or in women with previous VTE or thrombophilia is LMWH (Table 3-5). At the time of delivery, LMWH should be discontinued at least 24 hours before the induction of labor or cesarean delivery (or expected time of neuraxial anesthesia). Antithrombotic prophylaxis, where indicated (Table 3-5), should be continued for 6 weeks postpartum with prophylactic- or intermediate-dose LMWH or vitamin K antagonists (VKAs) targeted to an *international normalized ratio* (INR) 2.0-3.0.

Management: For women with acute VTE, adjusted-dose subcutaneous LMWH is recommended during pregnancy and continued for at least 6 weeks postpartum, for a minimum total duration of 3 months. For pregnant women with prior VTE, postpartum prophylaxis for 6 weeks is recommended, with prophylactic or intermediate-dose LMWH or VKAs targeted to INR 2.0-3.0. For pregnant women with low risk of recurrent VTE (eg, associated with transient risk factor unrelated to pregnancy or use of estrogen), antepartum clinical vigilance and postpartum prophylaxis is recommended. For pregnant women at moderate to high risk of recurrent VTE (eg, single unprovoked VTE, pregnancy- or estrogen-related VTE, or multiple prior unprovoked VTE not receiving long-term anticoagulation), antepartum prophylaxis with prophylactic or intermediate-dose LMWH and postpartum prophylaxis is recommended.

Pregnancy and thrombophilia, with and without thrombosis

Between 20% and 50% of all thromboembolic events that occur in pregnant women are associated with a thrombophilic disorder. Published studies indicate the relative risk of VTE associated with a thrombophilic disorder in pregnant women without a family history of VTE is increased about 34-fold in women who are homozygous for FVL mutation, eightfold in women heterozygous for FVL mutation, 26-fold for women homozygous for prothrombin G20210A gene mutation, and nearly sevenfold in women heterozygous for prothrombin G20210A mutation. The positive predictive value for pregnancy-associated thrombosis is 1:500 for the FVL mutation, 1:200 for prothrombin G20210A, and 4.6:100 for double heterozygotes of these mutations, assuming an overall thrombosis rate of 0.66/1,000 pregnancies. The positive predictive value was 1:113 for protein C deficiency and 1:2.8 for type I antithrombin deficiency. Regardless of presence of thrombophilia, a positive family history increases the risk for VTE two- to fourfold. Cohort studies in women with protein C deficiency (with no past VTE) and a positive family history have a 1.7% risk of developing a first antepartum or postpartum VTE, whereas women who are homozygous carriers for FVL and a positive family history have a 14% risk. Given the differing absolute risks of thrombosis, management 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 (Table 3-5).

Management: For pregnant women with no prior VTE and known to be homozygous for FVL or prothrombin 20210GA mutation, and a family history of VTE, antepartum prophylaxis with prophylactic- or intermediate-dose LMWH is recommended. For pregnant women with all other thrombophilias and no prior VTE with a family history of VTE, antepartum vigilance is recommended, and postpartum prophylaxis with prophylactic- or intermediate-dose LMWH or VKAs targeted to INR 2.0-3.0. For pregnant women with prior VTE, postpartum prophylaxis for 6 weeks with prophylactic- or intermediate-dose LMWH or VKAs targeted to INR 2.0-3.0 is recommended.

Thrombophilia and pregnancy complications

A number of pregnancy complications have been linked to thrombophilic states. Adverse pregnancy outcomes, however, are not uncommon in the general population, with 15% rates of miscarriage, and a 5% rate of two or more pregnancy losses. The association between thrombophilia and pregnancy loss has been confirmed in a number of

Table 3-5 American College of Chest Physicians guidelines for antithrombotic therapy in pregnancy.

Scenarios

Acute VTE

For pregnant women with *acute VTE*, adjusted-dose subcutaneous LMWH is recommended during pregnancy and continued for at least 6 weeks postpartum, for a minimum duration of 3 months.

For pregnant women, LMWH is recommended for antithrombotic therapy over adjusted-dose heparin and over vitamin K antagonists.

Prior Episode of VTE

For pregnant women with *prior VTE*, postpartum prophylaxis for 6 weeks with prophylactic- or intermediate-dose LMWH or vitamin K antagonists targeted at INR 2.0-3.0.

For pregnant women receiving adjusted-dose LMWH, LMWH should be stopped at least 24 hours before induction of labor, cesarean delivery, or neuraxial anesthesia.

For pregnant women with *low risk of recurrent VTE* (single episode VTE with transient risk factor unrelated to pregnancy or use of estrogen), clinical vigilance is recommended.

For women on long-term vitamin K antagonists prepregnancy, adjusted-dose LMWH or 75% of a therapeutic dose of LMWH is recommended during pregnancy and long-term postpartum.

For pregnant women with *moderate to high risk of recurrent VTE* (unprovoked pregnancy- or estrogen-related VTE or multiple unprovoked VTE on no long-term anticoagulation), antepartum prophylactic- or intermediate-dose LMWH is recommended.

No prior VTE

For pregnant women with *no prior VTE* who have homozygous factor V Leiden or the prothrombin 20210A mutation and a *family history* of VTE, antepartum prophylaxis with prophylactic- or intermediate-dose LMWH and postpartum prophylaxis for 6 weeks with prophylactic- or intermediate-dose LMWH or vitamin K antagonists targeted at INR 2.0 to 3.0 is recommended.

For pregnant women with *all other thrombophilias and no prior VTE* and *no family history* of VTE, antepartum and postpartum clinical vigilance is recommended.

For pregnant women with *all other thrombophilias and no prior VTE who have a family history* of VTE, antepartum clinical vigilance and postpartum prophylaxis with prophylactic- or intermediate- dose LMWH, or in women who are not protein C or S deficient, vitamin K antagonists targeted to INR 2.0-3.0 is recommended.

For pregnant women undergoing cesarean delivery without additional thrombosis risk factors, early mobilization but not thrombosis prophylaxis is recommended.

For pregnant women with *no prior VTE* who are known to be homozygous for factor V Leiden or the prothrombin 20210A mutation, but *no family history* of VTE, antepartum clinical vigilance and postpartum prophylaxis for 6 weeks with prophylactic- or intermediate-dose LMWH or vitamin K antagonists targeted to INR 2.0 to 3.0 is recommended.

For women at increased risk of VTE after cesarean delivery because of the presence of one major and at least two minor risk factors, pharmacologic thromboprophylaxis (prophylactic LMWH) is recommended. For mechanical prophylaxis (elastic stockings or intermittent pneumatic compression) for those with contraindications to anticoagulants while hospitalized.

Adapted from Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. Venous thromboembolism, thrombophilia antithrombotic therapy, and pregnancy. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (9th ed.). *Chest*. 2012;141(2 suppl):e691S–e736S.

INR = international normalized ratio; LMWH = low-molecular weight heparin; VTE = venous thromboembolism.

case-control studies for women with thrombophilia, but it has not been confirmed in the 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 previous VTE or VTE in pregnancy from the analyses, do not support significant risk of recurrent pregnancy loss (Table 3-6). Neither has there been any demonstrated association between thrombophilia and preeclampsia, placental abruption, or fetal growth restriction.

Table 3-6 American College of Chest Physicians guidelines for thrombophilia-related fetal loss.

Scenario	Comments
Thrombophilia and pregnancy complications	
For women with inherited thrombophilia and a history of pregnancy complications, antithrombotic therapy is not recommended.	For women with a history of pregnancy complications, screening for inherited thrombophilia is not recommended.
Antiphospholipid syndrome (APLAs)	
For women who fulfill the laboratory criteria for APLA syndrome and meet the clinical APLA criteria based on ≥3 pregnancy losses, antepartum administration of prophylactic- or intermediate-dose UFH or prophylactic LMWH combined with low-dose aspirin, 75–100 mg daily, is recommended.	For women with recurrent early pregnancy loss, ≥3 pregnancy losses before 10 weeks of gestation, screening for APLAs is recommended.
Paroxysmal nocturnal hemoglobinuria (PNH)	
For women with PNH, antepartum prophylactic- or intermediate-dose LMWH followed by 3 months postpartum prophylaxis is recommended.	For women with PNH, the use of eculizumab during pregnancy and postpartum may be of benefit, although safety and efficacy in pregnancy has not been established in clinical trials.
Hypofibrinogenemia	
For pregnant women with defective or deficient fibrinogen, fibrinogen replacement to a level of 100 mg/dL is recommended, beginning early in pregnancy, continuing during pregnancy, and for 6 weeks postpartum.	Fibrinogen replacement by cryoprecipitate or fibrinogen concentrate, where available, also may be effective in preventing bleeding.
Heparin-induced thrombocytopenia (HIT)	
In pregnant women with HIT, or in those with allergic reaction to heparin or LMWH, antithrombotic therapy with fondaparinux or lepirudin in place of heparin or LMWH, is recommended.	As safety has not been established in pregnancy, the use of dabigatran (a new oral direct thrombin inhibitor) and rivaroxaban or apixaban (new oral anti-Xa inhibitors) should be avoided.

Adapted from Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. Venous thromboembolism, thrombophilia, antithrombotic therapy, and pregnancy. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (9th ed.). *Chest*. 2012;141(2 suppl):e691S–e736S; Bornikova L, Peyvandi F, Allen G, Bernstein J, Manco-Johnson MJ. Fibrinogen replacement therapy for congenital fibrinogen deficiency. *J Thromb Haemost*. 2011;9:1687–1704; Brodsky RA. How I treat paroxysmal nocturnal hemoglobinuria. *Blood*. 2009;113:6522–6527.

LMWH = low molecular weight heparin; UFH = unfractionated heparin.

Management: 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.

Antiphospholipid antibody syndrome

The strongest evidence of an association between thrombophilia and fetal loss comes from studies in patients with APLAs. A diagnosis of APLA requires both laboratory and clinical criteria. The laboratory criteria require the presence of lupus anticoagulant or moderate to high titer antibodies to immunoglobulin (IgG) or immunoglobulin M (IgM) anticardiolipin (>99th percentile) or IgG or IgM β-2-glycoprotein I (>99th percentile) on two occasions at least 12 weeks apart; and the clinical criteria require ≥3 first trimester pregnancy losses, or ≥1 pregnancy loss after 10 weeks

gestation, or ≥1 premature birth before 34 weeks of gestation. The importance of β-2-glycoprotein I antibodies is less well established. Correlation of APLAs with fetal growth restriction or placental abruption remains controversial. Among women with recurrent fetal loss (≥3 miscarriages), 15% have APLA. In some studies, such women experience fetal loss at a rate approaching 90% in subsequent pregnancies, whereas in other older studies, normal outcomes may occur, even in the absence of treatment. 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 were placebo controlled, have examined the effect of treatment of women with APLAs with aspirin, heparin, or both. These studies generally have demonstrated an advantage of aspirin and heparin over either aspirin or heparin alone, although a

recent, 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.

Management: For women who fulfill laboratory and clinical criteria for APLAs, antepartum prophylactic- or intermediate-dose unfractionated heparin (UFH) or prophylactic LMWH combined with low-dose aspirin, 75-100 mg daily, is recommended. For women with two or more miscarriages but no APLA or thrombophilia, no antithrombotic prophylaxis is recommended (Table 3-6). APLA screening is recommended in women with recurrent pregnancy loss (≥ 3 miscarriages before 10 weeks), or with thrombophilia and recurrent pregnancy loss.

Paroxysmal nocturnal hemoglobinuria

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. The disease results from an acquired mutation of the X-linked phosphatidylinositol glycan complement class A (PIG-A) gene within the hematopoietic stem cell. The PIG-A mutation results in deficiency of glycosyl-GPI anchors which link antigens to the cell surface. In the case of the red cell, the absence of two GPI-linked complement regulatory genes, 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, which in pregnancy may occur at visceral sites, including the inferior vena cava (Budd Chiari), splenic, hepatic, and cerebral veins. Thrombotic risk correlates with expression of GPI-linked proteins on the surface of granulocytes. 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. There is an 8% maternal mortality rate in women with PNH, primarily related to postpartum thrombosis, and a 4% 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, antithrombotic therapy is recommended antepartum and 3 months postpartum. Recently, eculizumab, a monoclonal antibody that targets the terminal component of complement, C5, has greatly improved the treatment of PNH. Uncontrolled case series of its use in pregnant women with PNH suggest that eculizumab is safe in pregnancy when given from conception to delivery, and prevents PNH-induced pregnancy complications.

Management: For pregnant women with PNH, antepartum prophylactic- or intermediate-dose LMWH followed by 6 weeks postpartum prophylaxis is recommended. Eculizumab during pregnancy and postpartum may be of benefit (Table 3-6), but there are no clinical trials data to confirm this.

Hypofibrinogenemia

Fibrinogen abnormalities, including afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia may be associated with thrombotic and hemorrhagic pregnancy complications, including spontaneous abortion, placental abruption, and postpartum hemorrhage. Up to 30% of patients with congenital fibrinogen deficiency have thrombotic complications, most commonly first-trimester abortion, most common in those with afibrinogenemia, with less frequent and less severe complications 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 3-6). Several experts have suggested that fibrinogen replacement should be initiated as early as possible in pregnancy. Small case series indicate that a fibrinogen level >100 mg/dL is necessary to maintain pregnancy and avoid pregnancy complications, whereas a level of 100 mg/dL is necessary to prevent hemorrhage. Thus, the aim of treatment is to achieve fibrinogen levels of at least 60-100 mg/dL.

Management: For pregnant women with defective or deficient fibrinogen, fibrinogen replacement to achieve a level of 100 mg/dL beginning early in pregnancy, continuing during pregnancy, and for 6 weeks postpartum is recommended.

Anticoagulation 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 is LMWH or heparin, based on evidence-based guidelines recently updated by the ACCP (see the Bibliography). Among women with protein C or S deficiency, VKAs should be avoided. In women with valvular heart disease (see Table 3-7). VKAs should be avoided at least during weeks 6-12 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.

Management: In pregnant women, LMWH is the preferred antithrombotic agent.

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 hypoplasia or stippled epiphyses and limb hypoplasia. The frequency of these abnormalities is estimated to be between 0.6% and 6%. The teratogenic effects occur primarily following exposure to warfarin during weeks 6-12 of pregnancy, whereas warfarin is probably safe during the first 6 weeks of gestation. VKAs used at any time during pregnancy have been associated with rare central nervous system (CNS) 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 an 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.

Management: In pregnant women, LMWH is the preferred antithrombotic agent.

Heparin-induced thrombocytopenia

Heparin-induced thrombocytopenia (HIT) is an uncommon problem in pregnancy, but with only case series and anecdotal reports, the exact frequency is not known. Both heparin and LMWH can cause HIT in pregnancy. The mechanism by which HIT occurs is binding of antibodies directed against the heparin-platelet-factor 4 complex, so-called heparin-platelet factor 4 (HPF4) antibodies, which result in thrombocytopenia, usually 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 thrombocytopenia (HIT). In pregnant patients with HIT or in those who have severe allergic reactions to heparin, fondaparinux or the direct thrombin inhibitor lepirudin is considered to be the first-line therapy (Table 3-6). These agents, which appear to be safe and effective in pregnancy, must be stopped to allow safe anesthesia and delivery. Finally, data

are insufficient regarding the safety of the new oral anti-Xa inhibitors (eg, rivaroxaban) and oral thrombin inhibitors (eg, dabigatran) to recommend their use in pregnancy (Table 3-6).

Management: In pregnant women with HIT, or in those with allergic reaction to heparin or LMWH, antithrombotic therapy with fondaparinux or lepirudin in place of heparin or LMWH is recommended.

Heparin-associated osteoporosis

Prophylactic UFH is associated with a substantial risk of osteoporosis and a 2%-3% incidence of vertebral fractures when administered throughout pregnancy. Several reports suggest substantially less osteoporosis occurs in patients who receive LMWH; however, at least one randomized trial found no difference in bone density between low-dose UFH and LMWH. LMWH displays a better pharmacokinetic profile, and its use is associated with a five- to tenfold reduction in the incidence of HIT, as compared with UFH. Thus, LMWH is now the preferred agent for the prevention and treatment of VTE in pregnant patients. As pregnancy progresses and the volume of distribution increases for LMWH, however, dose adjustments may be required. Some recommend at the 36th week of gestation that LMWH be changed to UFH, because of the shorter half-life of the former in the setting of incipient parturition and the potential use of epidural anesthesia.

Management: In pregnant women, LMWH is recommended as the preferred antithrombotic agent.

Artificial 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. Debate continues, however, as to whether the benefit to the mother in prevention of valvular thrombosis offsets the risk of warfarin-induced embryopathy and neurodevelopmental abnormalities in the fetus. A systematic review of observational studies from 1996 to 1997 that assessed outcomes with various anticoagulants in women with prosthetic heart valves, found that VKAs were associated with the lowest risk of valve thrombosis and systemic embolism, with no difference in fetal wastage and bleeding between groups. The incidence of valve thrombosis was 3.9% and systemic embolism was 9.2% in women receiving VKAs during pregnancy, with heparin substituted for VKAs from weeks 6-12, as compared with a 33.3% risk of thromboembolic complications with UFH alone throughout pregnancy.

These findings are consistent with other retrospective studies demonstrating that VKAs are associated with a lower risk of valve thrombosis, maternal thromboembolism, and systemic emboli. LMWH has been used in pregnant patients with mechanical heart valves, although valvular thrombosis on this regimen has occurred with concerning frequency. A literature review of pregnant patients treated with LMWH found an incidence of valve thrombosis of 8.64% and an overall rate of thromboembolism of 12.35%, with the lowest rates in those in whom anti-Xa levels were measured. By contrast, a review of observational studies between 2000 and 2009 indicated the use of LMWH or UFH during the first trimester or at term was associated with a higher rate of valve thrombosis, 7.2%, and a higher rate of maternal thromboembolism, 13.4%. In women switched from VKA to LMWH during the first trimester of pregnancy, reported in case series and cohort studies between 1996 and 2006, valve thrombosis and maternal thromboembolism occurred in up to 22.4% of pregnancies, with the lowest risk associated with dose-adjusted LMWH compared with fixed-dose LMWH. Thus,

because there is no single optimal choice of anticoagulation for managing women with prosthetic heart valves during pregnancy, an individualized approach is recommended. Specifically, this recommendation is based on the balance of risks of maternal thrombosis versus fetal abnormalities, as developed by the AACP (Table 3-7), with stringent monitoring of both agents and the addition of aspirin in those at highest risk for thromboembolism.

Management: For pregnant women with prosthetic heart valves, three approaches are recommended: (i) adjusted-dose LMWH throughout pregnancy; (ii) adjusted-dose UFH throughout pregnancy; or (iii) 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 VTE, 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, 75-100 mg daily, is also recommended.

Table 3-7 American College of Chest Physicians guidelines for pregnant patients with artificial heart valves.

Scenario	Comments
Pregnant women with prosthetic heart valves	
For women with mechanical heart valves <i>one of the following</i> anticoagulant regimens is recommended:	All patients should be assessed for additional risks for thromboembolism, including valve type, position, and history of VTE, with the decision influenced by patient preference.
<ol style="list-style-type: none">1. Adjusted-dose bid LMWH throughout pregnancy, adjusted to achieve the manufacturer's peak anti-Xa LMWH 4 hours after injection; or2. Adjusted-dose UFH throughout pregnancy administered SC every 12 hours in doses adjusted to keep the mid-interval aPTT at least twice control or to attain an anti-Xa heparin level of 0.35–0.70 U/mL; or3. UFH or LMWH (as above) until the 13th week, with substitution by vitamin K antagonists until close to delivery when UFH or LMWH is resumed.	
Pregnant women with prosthetic valves at high risk of thromboembolism	
1. For pregnant women with prosthetic heart valves at very high risk of thromboembolism in whom concerns exist about the efficacy and safety of UFH or LMWH (eg, older generation prosthesis in the mitral position or history of thromboembolism), vitamin K antagonists are suggested throughout pregnancy with replacement by UFH or LMWH (as above) close to delivery.	This recommendation puts equal weight toward avoiding maternal and fetal complications.
2. For pregnant women with prosthetic valves at high risk of thromboembolism, the addition of low-dose aspirin (75-100 mg/d) to one of the regimens above is suggested.	

Adapted from Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. Venous thromboembolism, thrombophilia, antithrombotic therapy, and pregnancy. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (9th ed.). *Chest*. 2012;141(2 suppl):e691S-e736S.

aPTT = activated partial thromboplastin time; LMWH = low-molecular-weight heparin; SC = subcutaneous; UFH = unfractionated heparin; VTE = venous thromboembolism.

Hematologic health issues in the premenopausal woman

Bleeding in the premenopausal woman

Bleeding disorders in women are an underrecognized and undertreated condition. Hemophilia, the most widely known and studied bleeding disorder, is a disease of males. Women, however, are as equally 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 will review the most common gynecologic manifestations of bleeding as well as recommendations for the laboratory evaluation and management of women presenting with excessive bleeding.

Menorrhagia

The ACOG defines heavy menstrual bleeding (HMB), or menorrhagia, as menstrual bleeding lasting for >7 days or resulting in the loss of >80 cc of blood per menstrual cycle. HMB is a common health problem, occurring in as many as 10%-15% of women during their lifetime, and this excessive bleeding can lead to iron deficiency and chronic anemia. Women with HMB have significantly lower perceived general health and have a poorer quality of life in terms of their ability to fully participate in school, work, athletic, 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%-90% of patients and that bleeding disorders are common among women presenting with HMB. Therefore, it is imperative that physicians screen for underlying bleeding disorders when evaluating an adolescent or woman with HMB. A recent opinion issued by the Adolescent Health Committee supports screening for vWD in adolescents presenting with severe menorrhagia. This screening should be performed before the initiation of hormonal therapy, because oral contraceptives (OCs) may mask the diagnosis. 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 a study of 115 women 13-53 years of age, presenting for evaluation of HMB, adolescents and perimenopausal women were just as likely to have an underlying hemostatic defect as women 20-44 years of age. An international consensus statement published by experts

in the field of obstetrics and gynecology and hematology in 2009, states that an inherited bleeding disorder should be considered if any of the following indicators are present: (i) HMB since menarche, (ii) family history of a bleeding disorder, or (iii) a personal history of one or more additional bleeding symptoms.

As HMB is such a frequent problem, it would be cost-prohibitive to screen all women with HMB 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. Lightly stained products obtain a score of 1, moderately stained a score of 5, and soaked a score of 20. A total score of >100 per menstrual cycle is associated with menstrual blood loss of >80 mL (definition of menorrhagia). 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 >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 eight questions in four categories: (i) severity of HMB, (ii) family history of bleeding disorder, (iii) personal history of excessive bleeding, and (iv) history of treatment for anemia (Table 3-8). The screen is considered positive if an affirmative response is obtained in any one of the four categories. The sensitivity of this tool for underlying hemostatic defects in adult women is 89%, which increases to 93%-95% with a serum ferritin level of ≤20 ng/mL and a PBAC score of >185, respectively. 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.

There are few published trials regarding the management of HMB. In 2007, the NHLBI published guidelines on the diagnosis, evaluation, and management of vWD. The expert panel recommended that the first choice of therapy for HMB

Table 3-8 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?	See 7a. below
Q7a. Did you have bleeding problem after surgery?	1 = Yes 0 = No
Q8. Have you ever been pregnant?	See 8a. below
Q8a. Have you ever had a bleeding problem after delivery or after a miscarriage?	1 = Yes 0 = No

The screen is considered positive if an affirmative response is obtained in any one of the four categories covered by the eight questions, including (i) bleeding severity, (ii) family history of bleeding disorder, (iii) personal history of excessive bleeding, and (iv) 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, Faiz A, Heit JA, Kouides PA, Luke A, Stein SF, Byams V, Miller CH, Kulkarni R. Evaluation of a screening tool for bleeding disorders in a U.S. multisite cohort of women with menorrhagia. *Am J Obstet Gynecol.* 2011;204:e1-7; and Philipp CS, Faiz A, Dowling NF, Beckman M, Owens S, Ayers C, Bachmann G. Development of a screening tool for identifying women with menorrhagia for hemostatic evaluation. *Am J Obstet Gynecol.* 2008;198:e1-38.

should be combined OCs, with the levonorgestrel intrauterine device as second choice. Desmopressin (DDAVP), antifibrinolytics, and vWF concentrate are listed as alternative options for controlling HMB in women who desire pregnancy. The management of HMB in adolescents presents some additional challenges, as the etiology is often multifactorial, and patients or parents may be reluctant or unwilling to use the hormonal preparations recommended as first-line therapy.

Combined contraceptive agents 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 combined OCs 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 in 12 of 14 patients. Although these agents are generally well tolerated in adolescents, there may be great hesitancy, particularly in the families of young, sexually abstinent adolescents, to use them, and time should be allotted for thorough discussion and education. Extended cycling or continuous regimens of OCs 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 levonorgestrel-releasing intrauterine system (LNG-IUS, Mirena®) 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. These same investigators have reported on the use of LNG-IUS in five adolescents with bleeding disorders (16-19 years of age). Larger studies are needed to document expulsion rates and patterns of menstrual bleeding in young nulliparous women with these devices. 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. Other progestin-only contraceptives, such as depot medroxyprogesterone acetate (Depo-Provera®), progestin-only pills, and the Implanon® subcutaneous implant, also reduce endometrial proliferation and therefore menstrual blood loss. Insertion of the Implanon® implant might cause bleeding in a woman with a bleeding disorder, and the use of a preprocedure hemostatic agent should be considered.

Management: Females with HMB since menarche or a family or personal history of bleeding, should be screened for a bleeding disorder. For women with menorrhagia, combined OCs containing both estrogen and progestin, are recommended first-line therapy (Table 3-9). Alternative approaches include the levonorgestrel-releasing intrauterine system or

other progestin-only contraceptives, including Depo-Provera®, progestin-only pills, or subcutaneous implants.

Hemorrhagic ovarian cysts and endometriosis

The second most common reproductive tract manifestation is hemorrhagic ovarian cysts, which occur more commonly in women with vWD, platelet function defects, and other more 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, tranexamic acid, and clotting factor replacement have been used to manage hemorrhagic ovarian cysts. OCs, which reduce the likelihood of ovulation and increase clotting factors, are used to prevent recurrences (Table 3-9). Even among women with bleeding disorders but without

Table 3-9 Management of menorrhagia, ovarian cysts, and pregnancy in women with von Willebrand disease.

Menorrhagia and ovarian cysts in women with vWD	Pregnancy in women with vWD
Women with menorrhagia or abnormal vaginal bleeding should have a full gynecologic evaluation before therapy.	Women planning pregnancy should have a preconception evaluation with a hematologist and high-risk obstetrician, skilled in management of vWD.
In an adolescent or an adult woman who does not desire pregnancy but may desire future childbearing, the treatment of choice for menorrhagia is combined oral contraceptives. Factor replacement may be required for severe acute bleeding or blood loss until the above treatment is effective.	Women with vWF levels <50 IU/dL or a history of severe bleeding: 1. Should be referred to a center with high-risk obstetric capabilities and expertise in hemostasis. 2. Should receive prophylaxis with desmopressin or vWD concentrate before invasive procedures. 3. Should achieve vWF:RCo and FVIII levels of at least 50 IU/dL before delivery and up to 3-5 days afterward.
If a woman would otherwise be a suitable candidate for an intrauterine device, the second choice of therapy for menorrhagia is the levonorgestrel intrauterine system.	If vWF:RCo and FVIII levels can be monitored and maintained >50 IU/dL during labor and delivery, and no other coagulation defects are present, then neuraxial anesthesia may be considered.
For a woman who desires pregnancy, treatment with factor replacement, desmopression, or antifibrinolytics may be tried to control menorrhagia. Dilation and curettage is not usually effective to manage excessive uterine bleeding in women with vWD.	Because coagulation factors return to prepregnancy levels within 14-21 days after delivery, health care providers should be in close contact with a woman during the postpartum period.
If all efforts to reduce menorrhagia by factor replacement or hormonal therapies fail, then surgical intervention may be required. For women not desiring future pregnancy, uterine ablation or hysterectomy. For surgical procedures, factor to maintain vWF or VIII >50 IU/dL is recommended.	
In an adolescent or adult woman with acute hemorrhagic ovarian cysts , treatment of choice is replacement therapy.	
In an adolescent or adult woman who does not desire pregnancy but may desire future childbearing, the first choice for long-term prevention of hemorrhagic ovarian cysts should be combined oral contraceptives.	

Adapted from James AH, Kouides PA, Abdul-Kadir R, et al. Von Willebrand disease and other bleeding disorders in women: consensus on diagnosis and management from an international expert panel. *Am J Obstet Gynecol*. 2009;201:12 e11-18; James AH. Guidelines for bleeding disorders in women. *Thromb Res*. 2009;(123)(suppl 2):S124-128; National Heart, Lung, and Blood Institute. *The Diagnosis, Evaluation, and Management of von Willebrand Disease*. Bethesda MD: National Institute of Health. NIH Pub. No.08-5832;2007.

vWD = von Willebrand disease; vWF = von Willebrand factor; VWF:RCo = ristocetin cofactor activity.

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 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.

Management: For females who develop hemorrhagic ovarian cysts, clotting factor replacement alone or together with tranexamic acid is recommended for acute management, and OCs 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. Progestin-only agents are as effective as estrogen-progestin combination agents and are available in oral, intramuscular, intrauterine, and subdermal forms. These agents are generally well tolerated, but VTE remains a potential risk of these agents. Over 40 case-control studies, prospective cohort studies, and randomized trials of women using OC provide estimates of the risk of VTE to be two to threefold greater than in nonusers, although the absolute risk is low, 2-4 per 10,000 person-years of OC use. Several studies, however, indicate that the VTE risk in heterozygous carriers of the FVL or prothrombin G20210A is greater than would be expected if the risks were additive, that is, 28-50 per 10,000 woman-years of OC use. In women with protein C or antithrombin III deficiency, the absolute VTE risk with OC use is reported to be even higher, 400 per 10,000 patient-years of OC use.

Although the increased risk of thrombosis has been attributed to the estrogen component of contraception preparations, the exact biological mechanism was unclear for decades because OCs influence many hemostatic parameters. Estrogen increases procoagulants, such as Factor VIII, vWF, and fibrinogen, and decreases fibrinolytic activity

Table 3-10 Risk of VTE associated with hormonal contraceptives.

Contraceptive	Odds ratio	95% CI
COC 30 mcg, desogestrel	7.3	3.30-10.00
COC 30 mcg, 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, DeVito C, Marzuillo C, Boccia A, Villari P. Oral contraceptives and venous thromboembolism. *Drug Safety*. 2012;35:191-205; Ueng J, Douketis JD. Prevention and treatment of hormone-associated venous thromboembolism: a patient management approach. *Hematol Oncol Clin NA*. 2010;683-694; van Hylckama VA, Helmerhorst FM, Rosendaal FR. The venous risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control. *BMJ* 2009;339:62291; Lidegaard O, Nielsen LH, Skovlund CW, Lokkegaard E. Venous thrombosis in users of non-oral hormonal contraception: follow-up study, Denmark 2001-10. *Br Med J*. 2012;344:e2990. Epub 2012/05/12. van Hylckama VA, Helmerhorst FM, Rosendaal FR. The risk of deep venous thrombosis associated with injectable depot-medroxyprogesterone acetate contraceptives or a levonorgestrel intrauterine device. *Arteroscler Thromb Vasc Biol*. 2010;30:2297-300; van Vlijmen EF, Veeger NJ, Middeldorp S, Hamulyak K, Prins MH, Buller HR, Meijer K. Thrombotic risk during oral contraceptive use and pregnancy in women with factor V Leiden or prothrombin mutation: a rational approach to contraception. *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, Karp R, Raghavan V, Terrin N, Bauer KA, Zwicker JI. Assessing the risk of venous thromboembolic events in women taking progestin-only contraception: a meta-analysis. *BMJ*. 2012;345:34944. CI = confidence interval; COC = combined oral contraceptive; IUD = intrauterine device.

and natural anticoagulants, such as protein S. Evidence has now accumulated that the negative influence of OCs 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 combined OCs (desogestrel) are associated with higher VTE risk (Table 3-10). Progestin-only contraceptives appear to confer lower VTE risk, but there have been no clinical trials to confirm this. The vaginal ring, however, is associated with an increased risk of thrombosis relative to pills.

In addition to the small absolute risk of thrombosis with OCs, women may have underlying thromboembolic risk 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, whereas sickle cell disease may activate the coagulation system via sickled red cells and endothelial damage.

WHO has categorized a large number of medical conditions according to the level of risk associated with a variety of contraceptive agents. The four 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 OC are nonpreferred contraceptive choices—any known inherited thrombophilia. Women who are considered to have an unacceptable level of thromboembolic risk with OCs 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 OCs, but as category 1 or 2 with regard to progestin-only products (POC). This remains unconfirmed with the lack of any trial assessing safety or efficacy of POCs. In case-control studies investigating the association between oral POC and VTE, the risk of VTE was not significantly greater in POC users as compared with nonusers (Table 3-10) 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 nonhealthy POC users. Concern regarding a possible risk of VTE with POC may stem from reports that higher dose progestins, used for noncontraception purposes, have been associated with VTE. Furthermore, an international study reported a possible increase in stroke risk in women with hypertension using injectable POCs. Most recently, 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 levonorgestrel-releasing intrauterine system

(Mirena®). Thus, with a paucity of clinical trials, many controversies remain regarding optimal contraception in women at risk for thrombosis.

Management: For premenopausal females with thrombophilia seeking contraception, the potential VTE risks associated with combined OCs should be weighed against the risk of unplanned pregnancy. In the absence of definitive trials, it is recommended that premenopausal females with thrombophilia avoid combined OCs. If combined OCs are used, coadministered antithrombotic therapy (eg, warfarin or LMWH) may reduce VTE risk. These agents, however, are not without risks and their use in this setting remains unproven. Alternatively, progestin-only contraceptives or the levonorgestrel intrauterine system may provide lower VTE risk than with combined OCs, but this awaits clinical trials. For the present, rather than strictly contraindicating these agents, counseling regarding the interaction between risks may help women make an informed decision regarding contraception.

Conclusion

This chapter has summarized the most recent evidence-based guidelines available through the NHLBI, NHF, ACOG, ACCP, 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 will be 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.

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Hematopoietic growth factors

Gary H. Lyman and Murat O. Arcasoy

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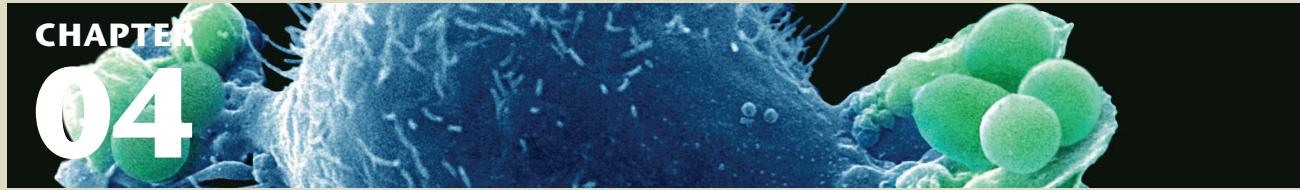
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CHAPTER
04



Hematopoietic growth factors

Gary H. Lyman and Murat O. Arcasoy

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Introduction

The hematopoietic growth factors (HGFs) and their receptors play essential roles in regulating hematopoiesis (see Chapter 2). 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; granulocytemacrophage 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. 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.

Myeloid growth factors

Granulocyte colony-stimulating factor (filgrastim, 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 (IL-1) and tumor necrosis factor (TNF).

Table 4-1 FDA-approved indications for filgrastim.

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

The biological effects of G-CSF are mediated through the G-CSF receptor expressed on both mature neutrophils and neutrophil progenitors. G-CSF knockouts in 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 myelogenous 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 7).

Available recombinant forms of G-CSF include filgrastim produced in *E. 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 methionine. Filgrastim is licensed for use in the United States and in many other countries (Table 4-1). 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

Conflict-of-interest disclosure: Dr. Lyman declares no competing financial interest. Dr. Arcasoy declares no competing financial interest.
Off-label drug use: Dr. Lyman: not applicable. Dr. Arcasoy: Epoetin alfa and darbepoetin alfa in myelodysplastic syndromes.

Table 4-2 FDA-approved indication for pegfilgrastim.

Reduce the risk of febrile neutropenia after myelosuppressive chemotherapy
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

biological activities and clinical benefits, including the duration of chemotherapy-induced severe neutropenia and occurrence of febrile neutropenia. 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 4-2.

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 IL-1 or TNF. GM-CSF promotes the growth of myeloid colony-forming cells (CFU-GM), 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, interleukin 3 (IL-3), and interleukin 5 (IL-5). Infants that are so affected have decreased alveolar macrophage function and accumulate surfactant in the alveoli.

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 U.S. Food and Drug Administration (FDA) (see Table 4-3).

Table 4-3 FDA-approved indications for GM-CSF sargramostim.

Reduce the risk of death due to infection in patients ≥ 55 years old undergoing induction chemotherapy for AML
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

AML = Acute myelogenous leukemia.

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 febrile neutropenia (temperature $>38.3^{\circ}\text{C}$ with neutrophils less than $0.5 \times 10^9/\text{L}$) in patients receiving cancer chemotherapy. Febrile neutropenia 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 febrile neutropenia was based on two 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 febrile neutropenia. 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 febrile neutropenia and documented infection by 50%. 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 febrile neutropenia 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 III double-blind, placebo-controlled clinical trial of primary prophylaxis with pegfilgrastim was conducted in patients with breast cancer receiving docetaxel 100 mg/m² 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 febrile neutropenia (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 4-2).

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 FN

GM-CSF is not FDA approved for the prevention of FN, and its use for this indication is now relatively uncommon. GM-CSF is approved to reduce the risk of death from infections in patients ≥ 55 years old undergoing induction therapy for AML (Table 4-3).

Clinical guidelines for the use of the myeloid growth factors

The American Society of Clinical Oncology (ASCO), the National Comprehensive Cancer Network (NCCN), and other organizations have developed guidelines for the use of myeloid growth factors to prevent FN. In brief, current ASCO guidelines (Table 4-4) include the following:

1. Primary prophylaxis is recommended for patients at high risk ($>20\%$) of FN due to age, medical history, disease

Table 4-4 ASCO guidelines.

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		Not to be used routinely
Therapy of Febrile Neutropenia	If high-risk for complications or poor clinical outcomes	Not to be used routinely
AML	Following induction therapy, patients >55 years old most likely to benefit After the completion of consolidation chemotherapy	Not to be used for priming effects
MDS		Consider in patients with severe neutropenia and recurrent infection
Acute Lymphocytic Leukemia	After the completion of initial chemotherapy or first post remission course	
Radiotherapy	Consider if receiving radiation therapy alone and prolonged delays are expected	Avoid in patients receiving 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 should be considered carefully

Smith TJ, Khatcheressian J, Lyman GH, et al. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. *J Clin Oncol*. 2006;24:187-205.

ALL = acute lymphocytic leukemia; AML = acute myelogenous leukemia; FN = febrile neutropenia; G-CSF = granulocyte colony-stimulating factor; MDS = myelodysplastic syndrome; NHL = Non-Hodgkins lymphoma.

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 a myeloid growth factors are listed in Table 4-5.

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 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 × 10⁹/L); use also should be considered for those >65 years old with pneumonia, hypotension, invasive fungal infections, or sepsis.

Acute myelogenous leukemia

Neutropenia, anemia, and thrombocytopenia are common presenting features of AML and also are important

Table 4-5 Risk factors for chemotherapy-associated neutropenia and its complications.

- Age greater than 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, Blayney DW, et al. Myeloid growth factors. *J Natl Comp Cancer Netw*. 2011;9:914-931.

complications in its treatment. There are many studies of the myeloid growth factors to sensitize leukemic cells to increase the effectiveness of chemotherapy and to prevent infectious complications. Although G-CSF and GM-CSF may shorten the duration of neutropenia during the induction phase of chemotherapy, neither consistently reduce 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 two 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 impact of G-CSF or GM-CSF on treatment response and survival has been observed.

Acute lymphoblastic leukemia

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 transplantation

Autologous peripheral blood stem cells now 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 (CXCR 4), expressed on hematopoietic progenitor cells, and its ligand chemokine ligand 12 (CXCL12, also known as stromal 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 Chapter 12, transplantation of autologous peripheral blood stem cells results in marrow engraftment

after myeloablative therapy. 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 2-4 days. Neutrophil recovery to $>0.5 \times 10^9/L$ is so rapid (median 11-14 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. Recent studies have shown that a CXCR4 antagonists called AMD3100 (also called plerixafor) act synergistically with G-CSF to yield greater numbers of CD34+ stem cells. AMD 3100 is now 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 transplantation

Currently, most transplant programs use peripheral blood stem cells in preference to bone marrow because of the ease of collection of peripheral blood stem cells and their more rapid neutrophil and platelet engraftment. When bone marrow transplantation is performed, a myeloid growth factor after bone marrow stem cell infusion significantly accelerates neutrophil recovery. In the pivotal study, patients undergoing autologous bone marrow transplantation for lymphoid malignancy were randomized to GM-CSF versus placebo after marrow infusion. Neutrophils recovered to $0.5 \times 10^9/L$ faster with GM-CSF. G-CSF had a similar effect. 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.

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, 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. Recently, the genetic and molecular causes for the inherited forms of severe chronic neutropenia have been discovered. Kostmann syndrome, autosomal recessive severe congenital neutropenia, is due to *HAX1* or *G6PC3* mutations. The *HAX1* gene encodes the HCLS1-associated protein X-1. Usually, it is diagnosed in an infant from a consanguineous marriage. The child will have neutrophils $<0.2 \times 10^9/L$, an arrest in myeloid maturation at the promyelocyte–myelocyte stage in the marrow, and recurrent infections. Sporadic and autosomal-dominant severe congenital neutropenia is a similar and more common condition and usually is attributable to mutations in the gene for neutrophil elastase, the *ELA* 2 or *ELANE* gene. Mutations in *G6PC3*, the gene encoding glucose-6-phosphatase, catalytic subunit 3, and *WAS*, the gene for the Wiskott–Aldrich protein, and rarely the gene for the G-CSF receptor (GCSFR or CD 114) may have a similar clinical phenotype. Cyclic neutropenia is another form of congenital neutropenia. These patients have neutrophil counts that oscillate on a 21-day cycle and have recurrent tissue infections during the periods of neutropenia. Cyclic neutropenia also is due to mutations in the narrow portion of the *ELA* 2 gene. Laboratory and clinical studies suggested that the neutropenia in all of these conditions is attributable to accelerated apoptosis of neutrophil precursors in the marrow.

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

Myelodysplasia is an acquired neoplastic hematopoietic stem cell disorder. Ineffective hematopoiesis results in decreased production of mature neutrophils, red cells, and platelets, and the neutrophils and platelets often have functional defects that further impair their ability to ward off bacterial infection or staunch bleeding. A number of clinical trials have investigated treatment of myelodysplasia with the HGFs. Treatment with G-CSF or GM-CSF can normalize the neutrophil count in most patients with myelodysplasia, but whether this translates into reduced mortality from bacterial or fungal infection is less clear. G-CSF or GM-CSF appears to enhance the effects of erythropoietin in the treatment of anemia and myelodysplastic patients. There is no convincing evidence at present that growth factor therapy accelerates progression from myelodysplasia to AML.

Other potential clinical uses of G-CSF

HIV

Neutropenia is common in advanced HIV infection. This complication was far more common before the availability of the highly effective antiviral drugs for this disease. Treatment with G-CSF promptly increases the neutrophil count to the normal range in most patients. A large multicenter trial randomized HIV-positive patients with a low CD4 count ($0.2 \times 10^9/L$) and absolute neutrophil count (ANC) $0.75-1.0 \times 10^9/L$ showed that G-CSF-treated patients (dose adjusted to increase the ANC to $2.0-10.0 \times 10^9/L$) 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 stem cell transplant recipients can transiently raise the peripheral neutrophil count to the normal range ($<2.0 \times 10^9/L$). Whether neutrophil transfusions will increase survival in patients with profound sustained neutropenia who have an active bacterial or fungal infection is under investigation.

Diabetes

A recent meta-analysis summarized the potential benefits of G-CSF as an adjunctive therapy for the treatment of diabetic foot infections. On the basis of an analysis of five 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 will 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, six 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 G-CSF-mobilized stem cells may improve cardiac function following myocardial infarction, presumably by stimulating angiogenesis. In one 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 chemo-radiotherapy. 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 the 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.

New versions 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 to 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 administered drug and also can neutralize the effects of the naturally produced, endogenous growth factors, thus worsening neutropenia.

The number of laboratories and biopharmaceutical companies producing myeloid growth factors also is rapidly increasing. Their products are molecularly similar to the approved products and are called “biosimilars.” Testing and introduction of biosimilars is proceeding rapidly.

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. 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 (BFU-e) and colony-forming unit-erythroid (CFU-e), indicating that commitment to erythroid lineage does not require EPO but rather that terminal differentiation of CFU-e into mature red blood cells depends on intact EPOR signaling.

Naturally occurring, dominant gain-of-function EPOR gene mutations that disrupt down-regulation 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 (VHL) 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 recombinant human erythropoietin (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 profile of these agents are comparable. The difference in the amount of posttranslational glycosylation of each product modulates the pharmacokinetic properties. These agents are produced

Table 4-6 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 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 two 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 by the FDA, but it is not available for distribution in the United States because of patent-related legal issues.

FDA-approved clinical uses of rhEPO

Chronic kidney disease

Normocytic, normochromic anemia associated with EPO deficiency occurs in the majority of patients with CKD

Table 4-7 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

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, although a cause-effect relationship is not established. 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 for 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 June 24, 2011, the FDA safety announcement indicated the following:

- Consider starting rhEPO treatment when hemoglobin level is <10 g/dL, without specifying how far below 10g/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 allo-immunization 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 50-100 U/kg administered subcutaneously once a week. Most patients respond to a regimen of 10,000 U/week. Darbepoetin alfa 60 µg every 2 weeks subcutaneously is an alternative regimen in predialysis patients.

For hemodialysis patients, the recommended initial dose of epoetin alfa is 50-100 U/kg three 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 µg/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 ≥ 100 ng/dL and the transferrin saturation is $\geq 20\%$. 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 will require red blood cell transfusions during the course of their illness. In this clinical setting, epoetin alfa and darbepoetin alfa exhibit efficacy to significantly increase hemoglobin and to reduce the requirement for red blood cell transfusions during chemotherapy. In a series of nine 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. Since 2010, prescribers and hospitals must enroll in and comply with a risk management program REMS (risk evaluation and mitigation strategy), 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. 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.

The typical starting dose of epoetin alfa is 150 U/kg subcutaneously three 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.

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 (B12 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 <10g/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 administered at the lowest dose possible.
- Available evidence does not identify hemoglobin levels $\geq 10\text{ g/dL}$ 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 6-8 weeks in nonresponders.
- rhEPO should be avoided in cancer patients not receiving concurrent chemotherapy, except for those with lower risk myelodysplastic syndromes (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 has decreased in the era of highly active antiretroviral therapy (HAART). 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, 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 $<0.2 \times 10^9/\text{L}$, 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 (100-200 U/kg three 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 (40-60,000 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.

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 pre-treatment hemoglobin of 10-13 g/dL, significantly fewer epoetin-treated patients (23%) required transfusions compared with a placebo group (45%). In the cohort with baseline hemoglobin of 13-15 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 and hemoglobin level 9.8-13.7 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 thrombo-prophylaxis, patients were randomized to preoperative epoetin alfa 600 U/kg for four 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 reported to facilitate autologous blood donation, although its routine use for this indication is not justified in clinical practice. Selected anemic patients who are willing to donate autologous blood or those who refuse 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 three different dosing regimens of epoetin alfa or placebo beginning 25–35 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 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 that 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 allo-immunization 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/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 non-responders. 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 has 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. The safety and efficacy findings of a randomized controlled trial (RED-HF) with an estimated enrollment of 2,600 patients are pending.

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 head-neck, breast, non-small-cell lung, uterine cervix, lymphoproliferative malignancies, 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 eight trials involved chemotherapy-treated patients, two trials included patients treated with radiotherapy only, and two trials involved patients with advanced cancer who did not receive antitumor therapy. In all eight trials, the target hemoglobin level during rhEPO treatment was >12 g/dL, higher than recommended. In two 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 hemoglobin decline >0.5-1.0 g/dL/week and low reticulocyte count <10/ $\times 10^9$ /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. A novel EPOR agonist peginesatide that does not cross-react with EPO antibodies has been used successfully in the treatment of some patients.

rhEPO biosimilars and other erythropoiesis-stimulating agents

The rationale for the development of epoetin biosimilars ("follow-on biologics") 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.

Pingesatide is a synthetic peptide-based erythropoiesis-stimulating agent (with no sequence similarity to EPO) that stimulates the EPOR dimer, activating 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 III clinical trials have been completed for the treatment of anemia in patients with CKD. FDA approval was granted 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 two trials involving predialysis CKD patients. The initial dose for patients on dialysis not currently receiving a rhEPO product is 0.04 mg/kg once a month given intravenously or subcutaneously. Prolonged half-life and once-monthly administration of peginesatide may offer potential advantages compared with rhEPO preparations, although the available clinical experience currently is relatively limited. Clinical trials with long-term follow-up and postmarketing surveillance information will be required to characterize relative clinical efficacy, potential long-term effects, and safety profile of peginesatide compared with rhEPO.

A novel class of erythropoiesis-stimulating agents in clinical development involves HIF stabilization by pharmacologic 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* (ESRD) 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. The disruption in mice of the gene encoding either TPO or *MPL* leads to severe thrombocytopenia due to reduced number of megakaryocytes.

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%-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 to increase platelet counts, durable responses as long as therapy is continued, and reduction in the need for other treatments led to FDA approval of both agents in 2008 (Table 4-8). 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 increases 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 IgG1 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 two 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

Table 4-8 FDA-approved indication for romiplostim and eltrombopag.

Treatment of thrombocytopenia in patients with chronic immune thrombocytopenia who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy

patients compared with 0% of placebo among splenectomized patients and 60% of romiplostim-treated patients compared with 4% placebo among nonsplenectomized 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 $\geq 50/\times 10^9/L$. The maximum weekly dose is 10 $\mu\text{g}/\text{kg}$. Treatment goal is to achieve and maintain a platelet count $\geq 50/\times 10^9/L$ 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 $\geq 50/\times 10^9/L$ at 6 weeks was 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 Caucasian 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 $\geq 50/\times 10^9/L$. 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%-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 reinstitute 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%-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 two patients with ITP and antiphospholipid antibodies. Kidney biopsy showed acute thrombotic microangiopathy and tubular injury in one 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. 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.

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. Eltrombopag was reported to improve platelet counts, anemia, or neutropenia in 44% of 25 patients with refractory aplastic anemia. 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.

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 six patients in the eltrombopag group compared with one patient in the placebo group. Five of the six patients treated with eltrombopag had platelet counts $>200/\times 10^9/L$. An association between an increased risk of thrombotic events and platelet count $\geq 200/\times 10^9/L$ 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 were reported to date. Studies investigating the efficacy and safety of eltrombopag for thrombocytopenia associated with chronic liver disease are ongoing.

Myelodysplastic syndromes

A phase I/II 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 two 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 less 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).

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Jecko Thachil, Lawrence A. Solberg, Jr., Marc J. Kahn and Keith R. McCrae

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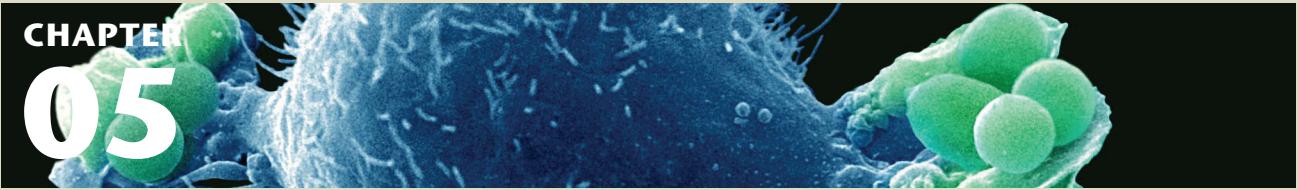
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CHAPTER
05



Iron metabolism, iron overload, and the porphyrias

Jecko Thachil, Lawrence A. Solberg, Jr., Marc J. Kahn, and Keith R. McCrae

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Introduction

Iron is a mineral required by mammalian cells for DNA synthesis, oxygen transport, and respiration. Iron's ability to accept and donate electrons allows it to shuttle between ferrous (Fe^{2+}) and ferric (Fe^{3+}) oxidation states and is essential for its participation in a number of enzymatic reactions. Despite the importance of iron to living cells, it also can be toxic. Iron catalyzes the formation of free radical ions, and therefore under physiologic states, it does not exist unbound to proteins or heme. Causes of iron overload include repeated blood transfusions, the ineffective erythropoiesis that characterizes certain chronic anemias, and mutations in a number of genes that lead to decreased production of or resistance to the iron regulatory hormone, hepcidin, which leads to increased iron absorption. This chapter focuses on iron metabolism in the normal host and in iron overload states, including hemochromatosis. Iron deficiency anemia will be discussed with the underproduction anemias in Chapter 6. This chapter also discusses the porphyrias as a model of disorders of synthesis of the iron-containing heme molecule.

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Regulation of iron homeostasis

The body's iron economy

The average content of iron in men is 35-45 mg/kg; menstruating women have lower amounts. Most of the iron in the body is present in the hemoglobin molecule in red blood cells. Each milliliter of red blood cells contains approximately 1 mg of iron (Figure 5-1). Men and women have approximately 2-1.5 g of erythrocyte iron, respectively. Iron is stored as ferritin or hemosiderin (partially denatured ferritin), predominantly in the macrophages of the spleen, bone marrow, and liver, but also in hepatocytes. At steady state, and in the absence of inflammation or severe iron overload, the serum ferritin level is a good reflection of total body iron stores. Total iron storage is approximately 1 g in men and 600 mg in women. Substantial amounts of iron are found as myoglobin in muscle, and much smaller amounts are found as cytochromes and other enzymes in all cells of the body. Only a tiny amount of iron is in the plasma bound to transferrin. Each molecule of transferrin can bind two molecules of ferric iron. Transferrin-bound iron constantly is turning over as iron is used, particularly developing red blood cells in the bone marrow. Dietary iron usually amounts to 15-25 mg daily, of which 5%-10% is absorbed. This proportion can be increased up to tenfold in states of iron deficiency. At steady-state conditions, the body requires 1-2 mg of iron daily to compensate for normal obligatory losses through physiological sloughing of epithelial cells, such as the epidermal cells and enterocytes, and trace amounts in urine and bile.

Iron metabolism is regulated carefully such that the amount of iron absorbed equals the amount of iron lost. There is no physiologically regulated pathway for iron excretion. During the past 15 years, much has been learned

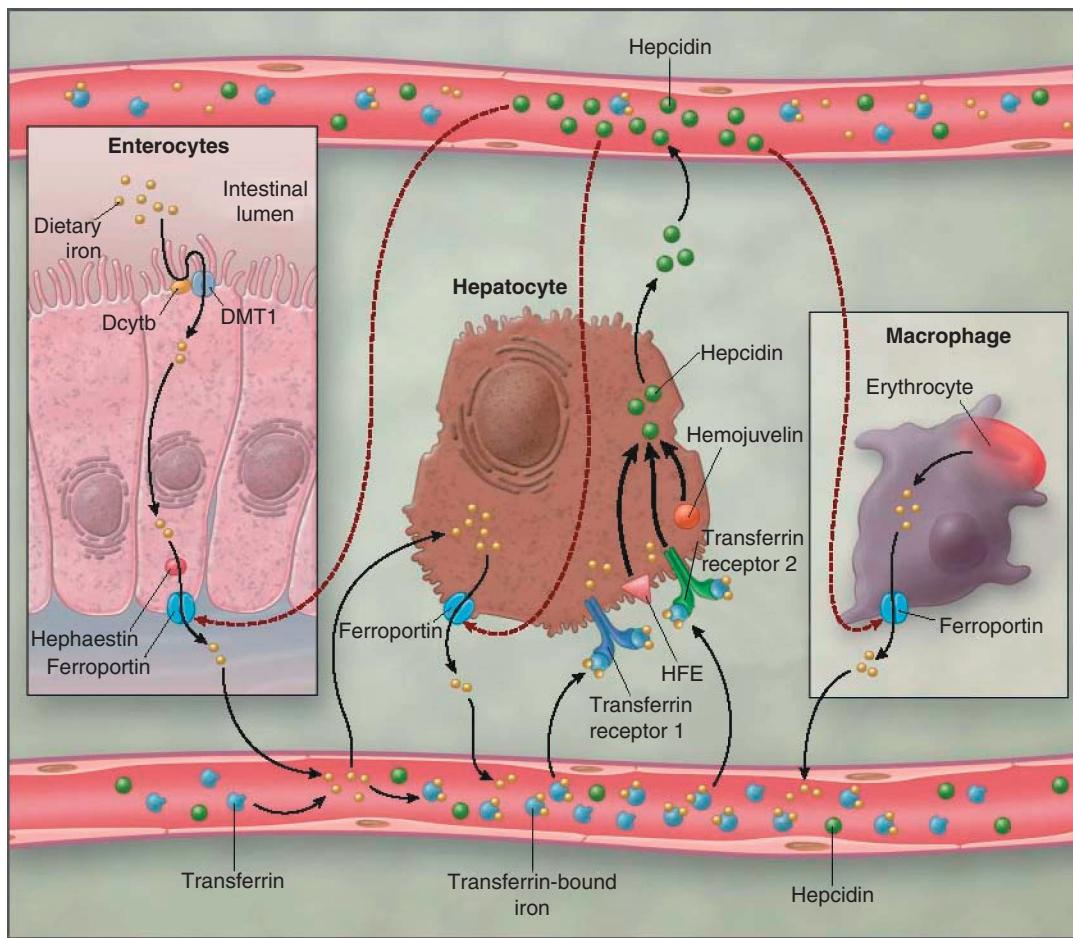


Figure 5-1 Regulators of iron balance. Dietary iron enters the enterocyte after being reduced to the ferrous state by duodenal cytochrome B (Dcytb) and being transported by divalent metal transporter 1 (DMT1). Hephaestin facilitates iron export by ferroportin. Hepatocytes take up either free or transferrin-bound iron and release it back into the circulation via the action of ferroportin. Ferroportin also releases iron from macrophages. Ferroportin-mediated release of iron is inhibited by hepcidin. With permission from Fleming RE, Bacon BR. Orchestration of iron homeostasis. *N Engl J Med.* 2005;352:1742, with permission.

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

In food, iron is found as inorganic iron and as heme (iron complexed to protoporphyrin IX). The typical diet consists of 90% inorganic iron and 10% heme iron, although diets in the industrial world can contain up to 50% heme iron because of the ingestion of iron-rich meats.

Heme iron is the most bioavailable iron, and its absorption tends to remain constant, regardless of dietary composition. In contrast, inorganic iron absorption is regulated and depends on other constituents of the diet, being enhanced by ascorbic acid and inhibited by such compounds as phytates and polyphenols in cereals and plants. The rate of iron absorption is influenced by a number of factors, including the body's iron stores, the degree of erythropoietic activity,

the concentration of hemoglobin in the blood, the blood oxygen content, and systemic inflammation. When iron stores are low or when there is an increased erythropoietic activity, anemia, or hypoxemia, iron absorption increase. Conversely, the physiologically appropriate response to iron overload is downregulation of intestinal iron; it is this downregulation of iron absorption that fails in patients with hereditary hemochromatosis and chronic iron-loading anemias.

Iron is absorbed in the intestine through two distinct pathways. One pathway exists for the absorption of inorganic iron (Figure 5-1), and a second pathway is for iron bound to heme. Despite its importance in Western diets, little is known about the identity of the molecules essential for heme iron absorption. Nonheme iron in the diet is largely in the form of ferric oxyhydroxides (ie, rust), but the intestinal epithelial cell apical iron importer, divalent metal transporter 1 (DMT1 or SLC11A2), transports only ferrous iron. Consequently, iron must be reduced to be absorbed. One protein that may facilitate this obligatory reduction is duodenal cytochrome B

Table 5-1 Major proteins involved in iron homeostasis.

Regulation of iron balance relies on the function of a number of key proteins. Among these are the following:
Ferritin, the major iron storage protein
Transferrin (Tf), the major transport protein for iron in the circulation
Transferrin receptor (TfR1), which is responsible for delivering iron from the plasma into erythroid precursors in the bone marrow and other cells of the body
Transferrin receptor (TfR2), which is expressed on hepatocytes and helps to regulate the expression of hepcidin
Iron responsive protein (IRP) -1 and -2, which regulate synthesis of a number of proteins, including apoferritin and TfR1
Duodenal cytochrome b (Dcytb), which reduces intestinal luminal ferric iron to the ferrous form that can be transported by DMT1
Divalent metal transporter 1 (DMT1), which transports ferrous iron from the gut lumen into the enterocyte
Hepcidin, a 25-amino acid peptide that regulates iron absorption and release of iron from macrophages
HFE, a protein mutated in the majority of cases of hemochromatosis that is expressed on hepatocytes and helps to regulate hepcidin production
Hemojuvelin (HJV), a protein that regulates hepcidin production in concert with TfR2 and HFE
Ferroportin (FPN1), the protein responsible for iron export out of enterocytes and macrophages to Tf in the plasma; ferroportin bound to hepcidin is rapidly internalized and degraded; also referred to as solute carrier family 40 member 1 (SLC40A1) and iron-regulated transporter 1, transports iron from the inside to the outside of a cell
Ceruloplasmin (CP), a plasma and macrophage protein whose ferroxidase activity enhances the export activity of macrophage ferroportin, primarily known as a copper-containing protein, also catalyzes the copper-dependent oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron, which facilitates iron transport by transferrin, which can transport only ferric iron
Hephaestin (HEPH), a ceruloplasmin analog whose ferroxidase activity enhances the export activity of enterocyte ferroportin, has extensive homology with ceruloplasmin, transports dietary iron from villous enterocytes in the small bowel into plasma, and also converts ferrous to ferric iron and facilitates iron efflux
Transmembrane protease serine S6 (TMPRSS6), a membrane-bound serine protease expressed by hepatocytes that is thought to downregulate BMP signaling in response to iron by cleaving HJV
Bone morphogenetic proteins (BMPs), a group of soluble factors that bind to cell surface receptors; one or several BMPs are involved in the hepatocyte response to serum iron
Sons of mothers against decapentaplegic (SMAD) proteins, a group of intracellular signal transduction and transcription factors that, among other functions, promote hepcidin gene transcription in response to iron in a BMP-dependent manner

(Dcytb), a heme-dependent ferrireductase. DMT1 mRNA expression is highly responsive to iron deficiency. Once transported across the apical border of the enterocyte, iron may be stored within the cell as ferritin until the cell senesces and sloughs off into the feces, or it may be transported across the basolateral membrane into the portal plasma by ferroportin. Ferroportin 1 (FPN1) is the only known iron exporter in mammals and, like DMT1, only transports ferrous iron. The transport activity of ferroportin expressed by the enterocyte is enhanced by the activity of the copper-dependent ferroxidases hephaestin (HEPH), which is a transmembrane protein associated with the enterocyte, and ceruloplasmin (CP), a homologous molecule that circulates in plasma. Regulation of the fate of intestinal intraepithelial iron depends on hepcidin, which binds to ferroportin, inducing its internalization and degradation, thus curbing egress of iron from the enterocyte. Thus, systemic iron absorption is tightly regulated by hepcidin and its modulation of iron export across the basolateral membrane of the duodenal enterocyte.

Cellular iron uptake and storage

Ferric iron released into the circulation binds transferrin and is transported to sites of iron use; each molecule of

transferrin binds two ferric iron atoms. Diferric transferrin (holotransferrin) binds to the transferrin receptor (TfR1) and enters cells by receptor-mediated endocytosis. The regulation of the synthesis of multiple proteins relevant to iron metabolism, including TfR1, DMT1, FPN1, and ferritin are controlled posttranscriptionally at the levels of mRNA stability and translation (Figure 5-2). The mRNAs for certain isoforms of these proteins contain iron response elements (IREs) that have a conserved nucleotide sequence with a stem loop structure that bind iron responsive proteins (IRPs)-1 and -2. The mRNAs for ferritin, DMT1, and FPN1 have IREs in the 5' untranslated region and the mRNA for TfR has multiple IREs in the 3' untranslated region. In low-iron states, IRP-1 lacks an iron-sulfur cluster and is in a conformation that allows it to bind to IREs and modulate iron metabolism. Likewise, intracellular heme induces degradation of IRP-2. In this manner, both iron and heme deficiency negatively regulate IRP activity. When IRPs binds to mRNAs with 5' IREs, they generally inhibit translation of the target mRNAs. When IRPs binds to 3' IREs, they generally stabilize the target mRNAs and increase mRNA abundance. Thus, when cytosolic iron is low, for example, there is decreased synthesis of ferritin and increased synthesis of TfR1.

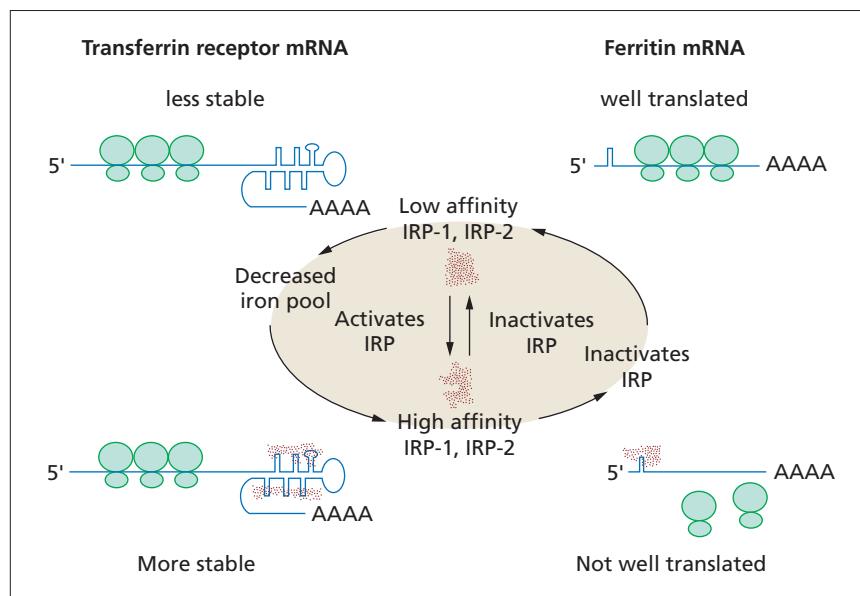


Figure 5-2 Coordinate regulation of transferrin receptor and ferritin synthesis by the iron regulatory proteins IRP-1 and IRP-2. Because both IRPs seem to respond to changes in cellular iron similarly and seem to bind to iron response elements (IREs) indistinguishably, only a single IRP is shown in the figure. Transferrin receptor synthesis is controlled by adjusting the amounts of cytoplasmic transferrin receptor messenger RNA (mRNA). The 3' untranslated region (3' UTR) of transferrin receptor mRNA contains five IREs. Binding of IRPs to the IREs in the 3' UTR retards cytoplasmic degradation, increasing the concentration of cytoplasmic transferrin receptor mRNA and the rate of transferrin receptor synthesis. With an increased number of cellular transferrin receptors, iron uptake is enhanced. By contrast, ferritin synthesis is controlled without changes in the amount of ferritin mRNA present by repressing translation of ferritin mRNA. The 5' untranslated regions (5' UTR) of ferritin mRNA contain a single IRE. Binding of an IRP to the IRE in the 5' UTR arrests translation of ferritin mRNA, so less ferritin is produced and iron sequestration is diminished. Redrawn with permission from Brittenham GM. Disorders of iron metabolism: deficiency and overload. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, eds. *Hematology: Basic Principles and Practice*. New York, NY: Churchill Livingstone; 1994:492-523.

Iron recycling

Most of the iron in the erythron couples with protoporphyrin to form the heme molecule. Heme forms a complex with the globin proteins, thus forming hemoglobin. Erythrocytes survive in the circulation for approximately 120 days, at which point the aging red blood cell is phagocytized by macrophages in the reticuloendothelial system. Within these macrophages, hemoglobin is catabolized, and iron is released to plasma transferrin by the action of ferroportin, which is the same as the iron export protein found within the gut enterocyte. When iron is once again released to plasma transferrin, the cycle repeats itself. Any iron not released to plasma (transferrin) may be stored within macrophages as ferritin or hemosiderin.

Regulation of systemic iron metabolism

Hepcidin, a 25–amino acid peptide, is the major regulator of iron absorption and storage. As alluded to, hepcidin regulates ferroportin activity by binding to an extracellular loop on the protein, leading to its internalization and degradation. Thus, elevated levels of hepcidin inhibit iron absorption from the gut and promote storage of iron within the hepatocyte and

macrophage. Hepcidin production can be increased more than 100-fold in inflammatory states, being induced by interleukin (IL)-6 and IL-1. Thus, like ferritin, hepcidin is an acute-phase reactant. Most, if not all, of the dysregulation of iron metabolism seen in the anemia of inflammation (anemia of chronic disease) can be attributed to this aspect of hepcidin regulation. As expected for a protein that negatively regulates iron absorption and recycling, hepcidin levels are downregulated by anemia and iron deficiency.

Regulation of hepcidin

The regulation of hepcidin is intimately associated with our understanding of the pathophysiology of hereditary hemochromatosis, in which defects in hepatocyte iron sensing lead to inappropriately low levels of hepcidin expression for the degree of iron stores. Although the details of the pathway are still evolving, it is clear that multiple hepatocyte cell surface proteins, including the hereditary hemochromatosis proteins HFE, hemajuvelin (HJV), and transferrin receptor-2 (TFR2) are involved in communicating the ambient transferrin saturation to a bone morphogenetic protein (BMP)/sons of mothers against decapentaplegic (SMAD)-dependent

signaling cascade that regulates hepcidin gene transcription. Iron-dependent BMP signaling is downregulated by the hepatocyte cell surface proteinease TMPRSS6 (transmembrane protease serine S6), which is thought to cleave HJV from the cell.

Hereditary hemochromatosis and other iron overload disorders

The term iron overload (hemosiderosis) is nonspecific and refers to a state of iron deposition in various body tissues or organs. Hemochromatosis refers to the clinical expression of iron-induced injury in affected body tissues. Hereditary hemochromatosis is a relatively common congenital cause of iron overload secondary to increased gastrointestinal absorption of iron at the level of the enterocyte. Other etiologies of hemochromatosis also exist and are discussed in the following sections (Table 5-2).

HFE hemochromatosis

Epidemiology and genetics

HFE hemochromatosis is the most common form of hereditary hemochromatosis. It is particularly prevalent in individuals of Northern European descent because of the presence of a common autosomal-recessive founder allele,

C282Y. In contrast, HFE hemochromatosis is distinctly uncommon in African Americans or Asians with iron overload. As will be discussed, tremendous variation exists between the genotypic and phenotypic expression of HFE hemochromatosis, even in individuals with the same mutations, because of the presence of genetic modifiers or environmental factors. Even in individuals homozygous for the C282Y allele, there is great debate regarding the penetrance of the disorder, depending on whether one defines the disorder on a biochemical, histopathological, or clinical basis; using the strictest clinical definition (ie, clinical evidence of iron-related organ dysfunction), it is likely that less than 25% of C282Y homozygotes will present with significant iron overload. A G-to-A mutation at nucleotide 845 of HFE leads to a cysteine-to-tyrosine substitution at amino acid position 282 (C282Y). Of Caucasians of European descent, 10%-15% are heterozygous and ~0.5% are homozygous for this mutation. C282Y homozygotes (C282Y/C282Y) account for 60%-90% of clinical cases of hereditary hemochromatosis. Although biochemical abnormalities, such as an elevated transferrin saturation or ferritin, rarely may be present in heterozygotes (C282Y/+), few will develop clinical features of iron overload in the absence of other environmental risk factors, such as alcoholic hepatitis.

A second mutation involves a G-to-C substitution at nucleotide 187 of HFE, leading to a histidine-to-aspartic-acid substitution at amino acid position 63 (H63D). Up to 30% of the Caucasian population are heterozygous for this more common variant allele. The H63D alteration is less penetrant than even the C282Y allele, and only a small minority of homozygotes (H63D/H63D) will develop clinical features of iron overload. Heterozygotes for the H63D mutation (H63D/WT) rarely develop biochemical or clinical evidence of iron overload.

Compound heterozygotes for the two mutations (C282Y/H63D) occasionally may develop mild iron overload and should be evaluated for coexisting risk factors if hemochromatosis is expressed clinically. In the United States, 15%-30% of patients with clinical hemochromatosis have no identifiable HFE mutation. The prevalence of different genotypic combinations among clinically affected individuals is listed in Table 5-3.

Although homozygosity for the C282Y allele accounts for up to 90% of clinical hereditary hemochromatosis, there remains much debate concerning the true phenotypic penetrance of HFE mutations. In a population screening study, approximately 50% of all homozygotes for the C282Y mutation developed phenotypic expression consistent with the disease, typically by the age of 60. In a pedigree study that investigated homozygous family members of known hemochromatosis probands, 85% of males and 65% of females had biochemical evidence of iron overload.

Table 5-2 Causes of hemochromatosis and iron overload.

1. Hereditary conditions affecting the hepcidin/ferroportin axis

- HFE hemochromatosis in Caucasians
- TPR2 hemochromatosis
- Hemojuvelin hemochromatosis
- Hepcidin hemochromatosis
- Ferroportin disease

2. Conditions of ineffective erythropoiesis

- β-thalassemia major and intermedia
- Hemoglobin E/β-thalassemia
- Hemoglobin H disease
- Congenital dyserythropoietic anemias
- Hereditary and acquired sideroblastic anemias

3. Multiple blood transfusions

- Aplastic anemia
- Diamond-Blackfan anemia
- Thalassemia major
- Sickle cell anemia
- Myelodysplasia

4. Other hereditary conditions

- African iron overload
- Melanesian iron overload
- Aceruloplasminemia
- Atransferrinemia

Table 5-3 Prevalence HFE genotypes among patients with hereditary hemochromatosis.

Genotype	Prevalence among patients with hereditary hemochromatosis
C282Y/C282Y	60%-90%
C282Y/H63D	0%-10%
C282Y/WT	Rare
H63D/H63D	0%-4%
H63D/WT	Rare
WT/WT	15%-30%

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.

Adapted from Cogswell ME, Burke W, McDonnell SM, Franks AL. Screening for hemochromatosis. A public health perspective. *Am J Prev Med*. 1999;2:134-140.

WT = wild type.

Despite these findings, only 38% of males and 10% of females had disease-related symptoms, and only 15% had fibrosis or cirrhosis on liver biopsy. With increasing age, disease-related morbidity increased, especially in homozygous men older than 40 years of age. Another study suggested that the clinical penetrance of homozygous hereditary hemochromatosis is much lower than previously predicted. In this study, the most common symptoms of hereditary hemochromatosis were no more prevalent in homozygotes (C282Y/C282Y) than in an unaffected control population. The penetrance of individuals homozygous for the C282Y mutation was estimated to be <1%. Other subsequent studies also suggested a low clinical penetrance of homozygous hemochromatosis. The true clinical penetrance of homozygous hemochromatosis is uncertain but probably lies somewhere between 1% and 25%. Much of the variability in these estimates involves the different populations studied (blood donors vs. preventive care clinics vs. general population vs. family members of affected probands) and how clinical penetrance is 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 of the disease. Patients often present to hematologists for evaluation of abnormal iron studies initially identified during health physicals, as part of screening when affected relatives are identified, or when iron panels are drawn for a variety of other indications. Early diagnosis is essential to alter the disease course and avoid end-organ complications. The clinical presentation is varied and often consists

of nonspecific findings, such as chronic fatigue, weakness, nonspecific abdominal pain, arthralgias, and mild elevation of liver enzymes, all of which may be noted years before the correct diagnosis is made. Patients may be diagnosed mistakenly with seronegative arthritis or pseudogout. Endocrine organs commonly are affected. Diabetes, hypothyroidism, and gonadal failure may occur. Both the mechanical and conduction systems of the heart can be affected, and patients may present with heart failure or arrhythmias. An increased frequency of depression has been noted. Iron-induced liver damage remains the most recognized and feared complication of untreated disease.

The transferrin saturation in patients with homozygous hemochromatosis is higher than in normal individuals, but it shows considerable variability. A transferrin saturation >50% in males or >45% in females should be followed by a repeat measurement, preferably in a fasting state, and the addition of serum ferritin. Ferritin values, although imperfect, are a surrogate marker for total body iron stores. Ferritin values can be elevated in several conditions other than iron overload, including the metabolic syndrome, inflammatory conditions, acute or chronic hepatitis, alcoholic liver disease, and the hyperferritinemia-cataract syndrome. In a population-based screening program performed through the U.S. Centers for Disease Control and Prevention, only 11%-22% of individuals with an elevated serum transferrin saturation had a concurrent elevation in serum ferritin (Figure 5-3).

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

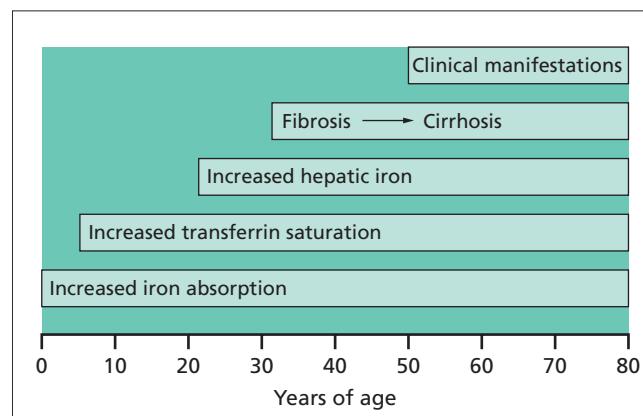


Figure 5-3 The natural history of hemochromatosis. An increase in the percent saturation of transferrin can be detected in children homozygous for hemochromatosis. Increased liver iron stores generally can be detected in homozygous men by the end of the second decade. The serum ferritin concentration 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.

been ruled out or if genetically or if clinically proven affected family members exist. Before genotyping is performed, a detailed discussion with the patient concerning the possible clinical, emotional, and financial implications of making such a diagnosis should be undertaken.

A liver biopsy is the gold standard in making the diagnosis of iron overload. Liver biopsy provides information on iron content, iron distribution, and whether fibrosis or cirrhosis has developed. Liver biopsy has been recommended for HFEC282Y homozygotes with abnormal liver function tests or ferritin >1,000 ng/mL to evaluate for cirrhosis. Liver biopsy also has been considered if a strong suspicion of pronounced iron overload exists despite a negative evaluation for HFE mutations or other primary or secondary causes. Cirrhosis is rare on liver biopsy if the serum ferritin is <1,000 ng/mL regardless of age or serum liver enzyme levels. In hereditary hemochromatosis, if liver biopsy is performed, the distribution of iron is primarily within hepatocytes (parenchymal), sparing Kupffer cells. A Perls stain of grade 3 or 4, a hepatic iron concentration of >80 mmol/g (4.5 mg/g) dry weight, or a hepatic iron index score ≥ 1.9 (= hepatic iron in mmol/g \div patient age) all confirm the presence of increased body iron stores. An additional method of estimating total body storage iron is by phlebotomy. If >4 g of iron (~16 units of blood) can be mobilized by phlebotomy without the patient becoming iron deficient, body iron stores are at least four times greater than normal. Liver biopsy is being performed less frequently in the evaluation of hereditary hemochromatosis now that confirmatory genotyping has become readily available.

Techniques including T2* hepatic magnetic resonance imaging (MRI) or superconducting quantum interference device (SQUID) susceptometry are other noninvasive methods currently being investigated. MRI for quantifying the amount of iron deposition in the liver and heart is becoming available in increasing numbers of centers. SQUID is available in only a few centers.

Treatment

Prompt and aggressive treatment before end-organ complications occur is the key to management. The life expectancy of patients can be normal if this goal is met. Phlebotomy is the key therapeutic modality. Removal of 1 unit of blood (200-250 mg iron) should be initiated at weekly intervals until ferritin levels decrease to 20-50 ng/mL, provided the hematocrit is maintained above 33%-35%. Normal adults will become iron deficient after four to six phlebotomies on such a program because the typical 1 g of iron stores would be depleted. Patients with 4 g of storage iron will not become iron deficient until 16-20 phlebotomies have been performed. In more advanced cases, total body iron burden can

be >20 g, requiring weekly phlebotomy for >1 year. After the ferritin value has decreased to 20-50 ng/mL, lifelong maintenance phlebotomy at less frequent intervals typically is required. Most often, phlebotomy is required every 2-4 months to maintain the ferritin level in the target range. In addition to ferritin, some practitioners monitor the hemoglobin and red blood cell parameters, such as red cell distribution width (RDW) or the cellular hemoglobin of the reticulocyte (CHr), to ascertain borderline iron deficient erythropoiesis as an objective, functional assessment of systemic iron status.

Therapeutic phlebotomy is often effective at improving a patient's overall sense of well-being, including resolving fatigue and malaise, normalizing skin pigmentation, and reducing elevated liver enzymes. The effect of phlebotomy on improving arthralgias, diabetes, and hypogonadism is less pronounced. Phlebotomy may not reverse cirrhosis or its attendant risk for hepatocellular carcinoma.

Phlebotomy usually is not indicated and only infrequently performed during adolescence. If an isolated increase in transferrin saturation is identified during screening, ferritin values should be monitored at 3- to 6-month intervals. Phlebotomy should be initiated only when ferritin values reach >300 ng/mL in males or >200 ng/mL in nonpregnant females of reproductive age. Avoidance of alcohol and exogenous medicinal iron or iron-containing vitamins should be stressed. Dietary change aimed at avoiding iron-containing foods is often impractical and not necessary as long as patients are compliant with phlebotomy. Patients should be warned about the risks of eating raw seafood because the incidence of *Vibrio vulnificus* and *Yersinia enterocolitica* infections is increased. Patients with iron overload are also at risk for mucormycosis, especially as they begin chelation therapy. Treatment of cardiac, hepatic, and other complications of iron overload is essential. Liver transplantation has been performed in the setting of end-stage liver disease. Iron chelation should be considered if phlebotomy is contraindicated. As the disease advances and iron deposition goes untreated, hepatic fibrosis and cirrhosis may develop. 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 (AFP) may be employed to screen for hepatocellular carcinoma in at-risk individuals.

Screening

Controversy exists concerning the role of population screening for hereditary hemochromatosis. Currently, population-based screening is not recommended. Early screening of at-risk individuals or families should be stressed. Measurement of transferrin saturation and serum ferritin

concentration and genotyping of the HFE locus are appropriate screening methods in high-risk individuals. Issues of genetic discrimination need to be considered; some authorities recommend against genetic screening during childhood or adolescence. First-degree relatives of affected individuals should be screened for iron overload, but whether genotyping should be performed in these at-risk first-degree relatives to definitively rule in or rule out homozygous C282Y hemochromatosis is controversial. Both the pros and cons of genotypic screening should be discussed, and further evaluation should proceed based on the desires of each individual family member.

Other autosomal-recessive forms of hereditary hemochromatosis

Patients with HFE hemochromatosis rarely present before the fourth decade of life. Individuals with clinically significant iron overload in their 20s and 30s are more likely to have a severe, early onset form of autosomal-recessive hereditary hemochromatosis termed juvenile hemochromatosis because of recessive loss-of-function mutations in HJV or in hepcidin (encoded by the HAMP gene) itself. Individuals with juvenile hemochromatosis characteristically present with life-threatening heart failure and polyendocrinopathies (eg, hypogonadotropic hypogonadism and impaired glucose tolerance or diabetes mellitus) more so than liver dysfunction or other clinical manifestations of HFE hemochromatosis. These patients often require intensive management of cardiac complications during the initial phases of treatment, but like their less severely affected counterparts, they may recover fully with the institution of an aggressive iron-depletion regimen in the absence of irreversible organ dysfunction. Recessive mutations in TfR2 are quite rare, and the disease phenotype is largely indistinguishable clinically from HFE hemochromatosis other than that the disorder has near-complete penetrance in genetically at-risk individuals and may present at an earlier age. Like HFE hemochromatosis, a common feature of these disorders is a relative deficiency of hepcidin expression for the degree of iron overload; the severity of the disease phenotype roughly correlates with the magnitude of hepcidin deficiency. Neonatal hemochromatosis, a disorder that presents as perinatal liver failure and widespread systemic parenchymal iron deposition, is likely not a primary disorder of iron metabolism. Rather, it appears to be the secondary consequence of alloimmune hepatitis because of an unknown fetal-maternal antigen incompatibility similar to neonatal alloimmune hematologic cytophenias. Treatment with intravenous immunoglobulin (IVIg) beginning in midgestation has been shown to mitigate the severity of iron overload in newborns born to mothers with a prior affected child.

Ferroportin disease

Autosomal-dominant iron overload resulting from mutations of FPN1 is known as ferroportin disease. Some patients with FPN1 mutations have clinical and histopathological features largely similar to autosomal-recessive forms of hemochromatosis.

Characteristically, these individuals have mutations that affect the ability of hepcidin to bind or induce the degradation of FPN1, leading to a hepcidin-resistant phenotype. Other patients have a distinct phenotype in which serum ferritin often is increased in the presence of a low-normal transferrin saturation or hemoglobin. Histopathologically, these patients typically have substantial Kupffer cell iron storage early in the course of the disease. Notably, they often sustain an early decrease in serum iron and hemoglobin levels during phlebotomy, which may impair their tolerance of the treatment. Patients on this end of the ferroportin disease spectrum appear to have mutations that result in partial loss of the transport function of FPN1.

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 chronic anemias can be due to transfusion, chronic iron hyperabsorption, or both. Because there is no physiological way to excrete excess iron, blood transfusions inexorably lead to systemic iron accumulation. In individuals that are not transfused chronically, ineffective erythropoiesis, the intramedullary death of developing red blood cells, leads to inappropriately increased iron absorption because of the suppression of hepcidin production by hepatocytes through an as-yet incompletely understood mechanisms. Ineffective erythropoiesis can lead to significant iron-related morbidity even in the absence of periodic transfusion in patients with thalassemia intermedia, congenital dyserythropoietic anemias, and sideroblastic anemias. Blood transfusions are the predominant cause of iron overload in patients with transfused thalassemia major, aplastic anemia, pure red cell aplasia, myelodysplasia, and sickle cell anemia. Other than chronic, excessive supplementation, nutritional iron overload is distinctly unusual. Less severe forms of iron overload have been described in association with alcoholic cirrhosis, hepatitis C, nonalcoholic steatohepatitis, and porphyria cutanea tarda. In some of these disorders, the frequency of HFE mutations is higher than would be predicted and likely contributes to the risk of iron overload. Hereditary aceruloplasminemia may mimic hemochromatosis, but it is characterized by normal transferrin saturation and the presence of neurologic deficits. CP has ferroxidase activity that is important for the release of iron

from macrophages; therefore, patients with a mutated gene may accumulate excess iron.

Iron chelation therapy

The management of secondary causes of hemochromatosis is difficult. Anemia often exists, and red blood cell transfusion may be required, making phlebotomy impractical. Rarely, erythropoietin can be used to increase hematocrit values to a range safe for phlebotomy. Splenectomy may decrease transfusion requirements in thalassemia patients as well as in other selected cases of chronic hemolytic anemias. Therapy aimed at treating the underlying condition, as in aplastic anemia for example, should be strongly considered.

Deferoxamine is an effective iron-chelating agent used extensively in these conditions. When initiated early in the disease course, negative iron balance can be achieved and organ damage can be prevented. Deferoxamine is administered by nightly continuous subcutaneous infusion (up to 40 mg/kg) over an 8- to 12-hour period. Local skin complications of deferoxamine are frequent and include pain, swelling, and pruritus at the injection site. These complications can be minimized by rotation of injection sites, addition of hydrocortisone to the solution containing deferoxamine, antihistamines, or local measures. The potential ocular and auditory complications secondary to deferoxamine mandate annual audiologic and ophthalmologic evaluations. Chronic deferoxamine therapy is arduous, and poor compliance often diminishes potential therapeutic benefits. Most experience with deferoxamine has been in patients with thalassemia major or sickle cell anemia, which require chronic transfusion and thus have transfusional iron overload.

Deferasirox was the first oral iron chelator to receive approval from the U.S. Food and Drug Administration for the treatment of transfusion-related iron overload in adults and children <2 years of age. A phase III trial showed that 20–30 mg/kg of deferasirox daily was sufficient to reduce liver iron concentrations and serum ferritin levels. Potential adverse effects included nausea, vomiting, diarrhea, abdominal pain, skin rash, agranulocytosis, and increases in serum creatinine or liver function tests. It is recommended that serum creatinine, liver function tests, and a complete blood count be assessed before initiating therapy and monitored monthly thereafter to determine whether dose modification or discontinuation is necessary. Approximately one-third of deferasirox-treated patients experienced dose-dependent increases in serum creatinine, although most of the creatinine concentrations remained within normal range. Liver function should be monitored monthly, and if there is an unexplained, persistent, or progressive increase in serum transaminase levels, deferasirox should be interrupted or discontinued. The complete blood count should be monitored

as well, and deferasirox therapy should be interrupted if there is a decrease in the granulocyte count below the normal range. As with deferoxamine, cases of ocular and auditory disturbances have been reported.

In the United States, the most recently approved oral iron chelator is deferiprone, which is dispensed as an oral tablet dosed three times daily. As with the other chelators, there are significant side effects, including gastrointestinal upset, arthralgias, and elevated hepatic enzymes. Drug-induced neutropenia or agranulocytosis is a particular concern and requires weekly monitoring. Whereas optimal deferiprone treatment generally is not as effective in reducing liver iron concentration as deferoxamine or deferasirox, it appears to be particularly effective in reducing cardiac iron overload. Combination therapy with deferoxamine and deferiprone should be used for patients with significant cardiac iron loading, if possible. At present, other combination chelation therapies require further study.

Key points

- The absorption of iron is tightly regulated at the level of the enterocyte by hepcidin and ferroportin.
- Iron overload may be due to hereditary or acquired causes, or to repeated blood transfusions.
- The HFE^{C282Y/C282Y} genotype is the most common and most penetrant mutation, leading to clinical iron overload in hereditary hemochromatosis.
- The clinical penetrance of the HFE^{C282Y/C282Y} genotype is probably <30%.
- Clinical manifestations of iron overload are similar regardless of etiology.
- Phlebotomy to remove excess iron is the primary treatment for homozygous hemochromatosis.
- Iron chelation therapy with deferoxamine or desferrioxamine is an option when phlebotomy is not possible. Annual audiologic and ophthalmologic examinations are required in individuals treated with these agents.
- Some clinical manifestations of hemochromatosis are reversible, but cirrhosis and the risk for hepatocellular carcinoma are not.
- Population screening is controversial, but high-risk individuals should be screened.

The porphyrias

Introduction

The porphyrias are a group of metabolic disorders that arise from enzymatic defects in the heme biosynthetic pathway. The name *porphyria* is derived from the Greek word *porphuros*, or purple, which denotes the purple-red crystalline porphyrins that exhibit characteristic red fluorescence

on exposure to ultraviolet light. Porphyrins complex with iron to form heme, a molecule crucial for all biologic oxidation reactions. Although biosynthesis of heme occurs in all metabolically active cells containing mitochondria, the two predominant areas are the bone marrow (85%), where it is required for hemoglobin synthesis, and the liver (15%), where it helps in the formation of several enzymes, including cytochromes and hemoproteins. The clinical presentation of porphyria is varied, gaining itself the term *little imitator*.

Biochemistry

Heme synthesis starts with the condensation of glycine and succinyl CoA, into aminolevulinic acid (ALA) catalyzed by the enzyme aminolevulinic acid synthase (ALAS), in the mitochondria (Figure 5-4). Another six enzymes are involved in the reactions to convert ALA to protoporphyrin. In the final step of this enzymatic pathway, protoporphyrin is coupled with iron to create heme. Although these eight enzymatic steps are similar in the erythroid cells and liver, control of heme production differs between these two tissues, mainly due to the differences in rates of synthesis of ALA, coded by two different genes, *ALAS1* and *ALAS2*. In the liver, *ALAS1* is the rate-limiting enzyme and is downregulated by high levels of heme and upregulated by low heme levels. In erythroid cells, the rate is limited only by iron availability and not inhibited by heme. One practical implication of this difference is that heme can be used to treat an acute

hepatic porphyria attack. This also explains why drugs such as steroids, other chemicals, or stress can worsen hepatic porphyrias because they induce *ALAS1* along with cytochrome P450 enzymes. On the contrary, glucose can suppress gene expression of *ALAS1* (the glucose effect), accounting for the higher incidence of attacks while fasting and symptomatic response to glucose infusions. In erythroid cells, regulation is more complex with the *ALAS2* gene expressed 30-fold more than the hepatic enzyme.

Pathophysiology

The different types of porphyria arise from a deficiency of one of the eight enzymes in the heme biosynthetic pathway (see Table 5-4). This results in the accumulation of porphyrins and their precursors in a pattern specific to the enzyme involved, which is reflected in the specific clinical manifestations. During an acute attack, porphyrin precursors, ALA and porphobilinogen (PBG) are excreted in large quantities from the liver. These compounds are extremely neurotoxic especially for the autonomic and peripheral nervous systems, which lack blood-brain barrier protection. Although the blood-brain barrier protects the brain from these agents, they may still cause vascular injury and brain edema. The characteristic skin symptoms in porphyrias develop as a result of interaction of solar radiation and high amounts of circulating porphyrins, which accumulate in the skin. Once they absorb light, the porphyrin molecules emit energy and cause cell damage by peroxidation of cell membranes and

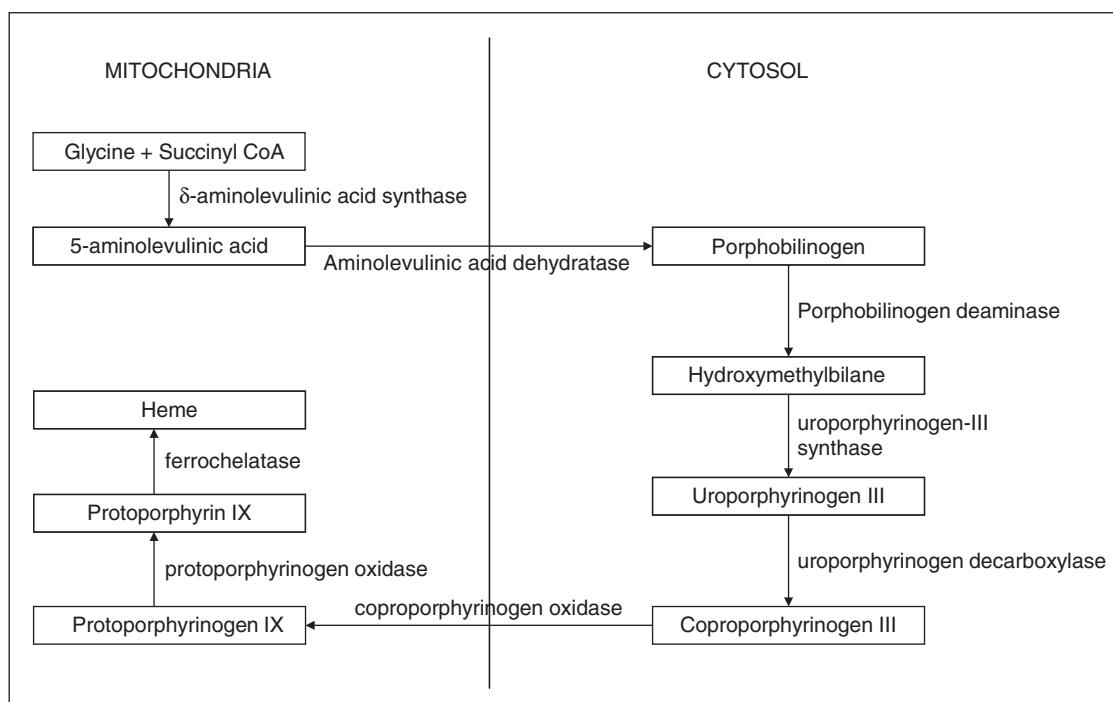


Figure 5-4 The heme biosynthetic pathway.

Table 5-4 Classification of porphyrias.

	Inheritance pattern	Enzyme affected	Organs involved	Symptoms	Comments
<i>Acute porphyrias</i>					
Acute intermittent porphyria	AD	Porphobilinogen deaminase/ hydroxymethylbilane synthase	Nervous system, liver	Neurovisceral	No cutaneous symptoms
Porphyria variegata	AD	Protoporphyrinogen oxidase	Nervous system, skin, liver	Neurovisceral Cutaneous	Common in South Africa
Hereditary coproporphyria	AD	Coproporphyrinogen oxidase	Nervous system, skin, liver	Neurovisceral Cutaneous	Skin lesions occur but not common
δ-ALA dehydratase porphyria	AR	ALA dehydratase	Nervous system, liver	Neurovisceral	Very rare, chronic neuropathy
<i>Non-acute porphyrias</i>					
Porphyria cutanea tarda	AD	Uroporphyrinogen decarboxylase	Skin, liver	Cutaneous	Sporadic and familial forms exist
Erythropoietic protoporphyrinia	AD	Ferrochelatase	Skin, red cells, liver	Cutaneous	Burning sensation in photosensitive areas
Congenital erythropoietic porphyria	AR	Uroporphyrinogen III synthase	Skin, red cells	Cutaneous hemolytic anemia	Erythrodontia bone changes
Hepatoerythropoietic porphyria	HZy	Uroporphyrinogen decarboxylase	Skin, red cells, liver	Cutaneous hemolytic anemia	Lab results similar to PCT

In all the conditions where liver is involved, chronic liver failure and hepatocellular carcinoma may develop.

AD = autosomal dominant; AR = autosomal recessive; XL = X-linked; HZy = homozygous defect; PCT = Porphyria cutanea tarda.

disruption of intracellular organelles. The principal site of light injury has been considered to be in the blood vessels of the papillary dermis. Skin biopsy in these cases is not informative and actually may cause harm.

Inheritance

Most forms of porphyria are autosomal dominant with incomplete penetrance, although some types are recessive. Rarely, X-linked or complex patterns of inheritance (compound heterozygote) are observed. Concurrent inheritance of more than one defect has been described in two situations: the Chester porphyria (acute intermittent porphyria and variegate porphyria) and dual porphyria (variegate porphyria and porphyria cutanea tarda). The penetrance of porphyrias varies with only about half of gene carriers demonstrating clinical manifestations, suggesting additional factors including environmental influences in determining the phenotype. This is exemplified by the fact that despite the prevalence of 1 in 10,000 for the individuals who carry the gene for acute intermittent porphyria, only about 10% actually will present with symptoms.

Classification

The porphyrias are classified as acute or nonacute (predominantly cutaneous) according to the presenting clinical features (Table 5-4). In the acute porphyrias, overproduction of all the porphyrins and porphyrin precursors proximal to the enzyme defect occurs. The precursors are excreted in large amounts due to the decreased activity of PBG deaminase (PBGD), either due to genetic mutation, as in acute intermittent porphyria, or by its feedback inhibition in variegate porphyria or hereditary coproporphyria. In the nonacute porphyrias, there is overproduction of all the porphyrins formed before the enzyme defect but not that of the porphyrin precursors, possibly because of a compensatory increase in the activity of the enzyme PBGD. Additional classification as hepatic or erythropoietic porphyria is based on the organ in which accumulation of porphyrins and their precursors primarily arises.

Acute porphyrias

Four different porphyrias present with acute features, including acute intermittent porphyria (the most common),

hereditary coproporphyria, variegate porphyria, and the extremely rare, δ -ALA dehydratase porphyria.

Acute intermittent porphyria

Acute intermittent porphyria (AIP; Swedish porphyria) results from the deficient activity of the PBGD. It affects about 1 in 75,000 people in European countries, except in northern Sweden, where it is more frequent (1 in 1,000) because of a founder effect. AIP is not associated with skin lesions. The mutations underlying AIP typically reduce the activity of PBGD by 50%, which does not always result in symptoms unless there is an induction of the rate-limiting hepatic enzyme *ALAS1*. The latter is induced by many non-genetic factors, including certain drugs, endocrine factors (explaining pubertal onset and relation to menstrual periods), and reduced calorie intake (linked with peroxisome proliferator-activated receptor γ coactivator-1 α). High levels of PBG contribute to the reddish or port-wine colored urine in AIP. Erythrocyte PBGD activity is decreased in most patients, although about 5% will have a genetic lesion only in liver cells, in which case detection of the PBGD mutation is confirmatory.

Other acute porphyrias

Variegate porphyria (VP; deficiency of protoporphyrinogen oxidase) and hereditary coproporphyria (HC; deficiency of coproporphyrinogen oxidase) differ from AIP in that they may present with cutaneous photosensitivity in addition to the neurovisceral symptoms. The cutaneous symptoms are related to the accumulation of photosensitizing porphyrins, which does not occur in AIP because the enzyme block in AIP is proximal to the production of porphyrins. Skin lesions develop in 60% of patients with VP and in about 5% with HC. These usually develop many days after sun exposure and typically occur on the back of the hands with easy fragility, blistering, and scarring occurring similar to patients with porphyria cutanea tarda. VP has a predominance in South Africa (characteristic mutation is Arg59Tryp), where it is the most common porphyria seen because of a founder effect from a mutation in a Dutch settler. δ -ALA dehydratase porphyria (ALAD) (doss or plumboporphyria) is the only acute porphyria inherited as an autosomal-recessive trait. Unlike the other acute porphyrias, the porphyrin precursor ALA and not PBG is increased substantially in the urine in this condition. Marked deficiency of ALA dehydratase in the absence of lead poisoning, suggests the diagnosis in mostly adolescent age-group patients. Chronic neuropathy can develop in this condition. A late-onset type is seen in association with myeloproliferative disorders. Recessive forms of AIP, VP,

and HC (harderoporphyria) also have been described in children with neurological symptoms and developmental delay as the predominant symptoms.

Clinical features of acute porphyria

The predominant symptoms of the acute porphyrias are neurovisceral. Attacks can begin with minor behavioral changes such as restlessness and insomnia and progress rapidly. One typical presentation is with abdominal pain followed by vomiting, constipation, and sometimes the development of bladder paresis. Pain in the back or extremities is a common feature. The differentiating features from surgical causes of acute abdomen include poor localization, absence of peritoneal signs or fever, and the absence of leukocytosis. The pathogenesis of pain is not yet well understood, although autonomic neuropathy, disturbances in smooth muscle function, intestinal angina, or lack of nitric oxide have all been suggested as possible reasons. In support of autonomic disturbances, the most common clinical sign is tachycardia often accompanied by hypertension, both signs of increased sympathetic activity. In progressive cases, the autonomic dysfunction can lead to arrhythmias and even occasionally cause cardiac arrest.

Peripheral neuropathy occurs in about 40% of cases of acute porphyria attacks, developing usually after the onset of the abdominal symptoms. Motor neuropathy is more common, similar to cases of Guillain-Barre syndrome, which is an important differential diagnosis. Mental disturbances, recurrent episodes, and abdominal symptoms are unusual with Guillain-Barre, however. Proximal muscles are predominantly affected in the porphyria neuropathy with upper-limb involvement in 50% of cases. Sensory neuropathy, when it occurs, has a bathing-trunk distribution, while cranial nerve involvement generally develops after the limbs and trunk are affected. Respiratory muscle weakness may develop with progressive disease and respiratory failure. Central nervous system involvement, with encephalopathy of varying severity, can develop with acute porphyria attacks. Cerebrospinal fluid examination is often normal in these cases. Seizures also may occur and often are associated with severe hyponatremia. This metabolic disturbance is secondary to a syndrome of inappropriate antidiuretic hormone secretion, gastrointestinal loss, and dehydration. Early imaging in neurological porphyria has demonstrated changes consistent with posterior reversible encephalopathy syndrome, linked to acute hypertensive episodes. The mechanism of neural damage in acute porphyrias is not understood although vasospasm resulting from decreased nitrous oxide production by nitrous oxide synthase (hemoprotein) or neurotoxicity from porphyrin precursors after its uptake into cells have

been suggested. ALA also can interact with γ -aminobutyric acid receptors. Dramatic response of acute attacks to liver transplantation supports the hypothesis that heme precursors from the liver cause the neurologic manifestations.

Psychiatric disturbances are features of acute attack of porphyria. These include depression, hallucinations, and delirium while frank psychosis also may occur. Many of these patients may be described as having a psychiatric disorder, the best example being the longest serving British sovereign, King George III. Nonspecific symptoms like fatigue also are common in acute porphyria with reports of up to 50% of the patients being affected by this symptom.

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 in these cases, regular screening using liver ultrasound or other methods is advisable in all adult patients. Chronic renal impairment may develop in acute porphyrias because of hypertension, although repeated vasospasms during the recurrent attacks also have been implicated.

Acute attacks are much more common in women and during the second to fourth decades, occurring rarely before puberty and after menopause. Menstrual cycles are a common precipitant of the acute attack, with recurrent attacks described typically in the late luteal phase (progesterone implicated in increased heme catabolism). Although oral contraceptive preparations aggravate the attacks, postmenopausal hormone replacement therapy does not seem to have a triggering effect. Other common aggravating factors are fasting, alcohol intake (inhibits many enzymes in heme biosynthesis pathway and also induces cytochrome P-450), and infection (fever induces heme oxygenase, decreased food intake, and antibiotics) in addition to several drugs.

Drugs and porphyrias

Acute porphyrias have been termed pharmacogenetic diseases because many drugs can precipitate attacks. The different mechanisms include induction of hepatic cytochrome P450 and inhibition of heme synthesis. Because heme biosynthesis is a complex biosynthetic pathway, many drugs may be anticipated to trigger an acute attack. A list of safe and unsafe drugs has been compiled into databases such as those at <http://www.porphyria-europe.com> and <http://www.drugs-porphyria.org>. Some drugs definitely are contraindicated, but many others are only potentially dangerous and the risk versus benefit for the use of these drugs should be considered in each situation.

Diagnosis of acute porphyria

The starting point is to suspect acute porphyria. Although certain symptoms like abdominal pain in those with reddish urine or muscle weakness typically occur after the start of a new drug or an oral contraceptive pill approximately one-tenth of the patients may not have any abdominal symptoms at all. Because delayed treatment can result in neurologic damage and sometime fatal consequences, a strong suspicion and early diagnosis is crucial.

There are two stages to diagnosing an acute neuropathic porphyria. The first step is to obtain evidence that there is an ongoing episode of one of the four acute neuropathic porphyrias (AIP, VP, HC, or ALAD). The fundamental test for this is a correctly collected 24-hour urine test. The second step is to determine the subtype of acute porphyria. This step involves a review of 24-hour urine, stool, and selected blood tests (eg, red cell enzyme determinations for PBG deaminase). The clinical status of the patient is important as well because if a patient is hospitalized and critically ill, in addition to definitive 24-hour quantitative urine tests, more rapid qualitative screening tests should be ordered. These qualitative tests may not be readily available, and empiric therapy might have to be started for critically ill patients. Clinicians must understand the ordering system of the reference laboratory with which their hospital or clinic works to ensure that appropriate tests are ordered and collected correctly. For example, some reference laboratories include the testing for the porphyrin precursor, PBG, in a 24-hour urine study, whereas others do not and the PBG must be ordered separately. The same applies to ALA determinations. If one is seeing a suspected acute porphyria patient for the first time, 24-hour determinations of both PBG and ALA should be undertaken to exclude the rare ALAD presentation.

In this context, it is worth noting that one common clinical circumstance encountered by community physicians or hematologists-oncologists is the observation of elevated 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 such patients will have normal PBG levels in the midst of their symptoms—a finding that excludes neuropathic porphyria, if the urine is collected correctly. These patients often arrive at referral centers suspected of having HC because of elevated urinary coproporphyrins but with no documentation of PBG levels in urine specimens. Another prevalent outpatient clinical circumstance is that some patients have secondary gain, and all of their prior 24-hour urine tests were collected when they were not having symptoms. In acute porphyria, PBG levels can normalize between attacks. In these instances, the clinician should instruct the patient to collect a 24-hour urine sample at a time the patient

determines he or she is experiencing clinical symptoms. True attacks of acute neuropathic porphyria are diagnosed easily and have high abnormal 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 in the index case to determine the subtype of acute porphyria are undertaken. Patients with VP and HC have characteristic 24-hour stool findings even between attacks. Biochemical confirmation of the type of acute porphyria can be done 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 engaged for familial studies.

Differential diagnosis of acute porphyria includes lead toxicity (where abdominal pain and neuropathy can coexist) and paroxysmal nocturnal hemoglobinuria (where abdominal pain and discoloured urine occur in the absence of peripheral neuropathy). The combination of peripheral neuropathy with central nervous system involvement is unusual in other conditions and should alert the possibility of porphyria as the likely diagnosis. Hereditary tyrosinemia type 1, which develops as a result of accumulation of succinyl acetone, an inhibitor of ALA dehydratase, can present in children with symptoms resembling acute porphyria.

Treatment of acute porphyria

Patients who present with acute porphyria attacks should be hospitalized. All contraindicated drugs should be stopped or avoided. A multidisciplinary approach should be considered for all patients because the clinical manifestations encompass multiple organ systems. Mild attacks, without symptoms like severe abdominal pain, neuropathy, and hyponatremia, may be treated initially with high carbohydrate intake of 2,000 kcal/24 hours orally or through a nasogastric tube. If this cannot be tolerated, intravenous 10% dextrose should be given targeting at least 300 g/day glucose. Precaution should be taken to avoid larger quantities of glucose, which may lead to hyponatremia. Opioids and phenothiazines can be given if necessary. Propranolol can be used for tachycardia and hypertension.

Severe attacks require treatment with intravenous hemin. Hemin binds to hemopexin and albumin in plasma and is taken up by the liver where it suppresses the ALAS. This agent should be started early in the clinical course for better clinical

outcome. The standard regimen is 1-4 mg/kg once daily of heme in the form of lyophilized hematin, reconstituted with human albumin to avoid thrombophlebitis (Panhematin, Lundbeck Pharmaceuticals, United States) and infused daily for 3-14 days, or heme arginate (Orphan Europe), infused daily for 4 days. Hematin is safe in renal impairment. Adverse effects with this drug include fever, hemolysis and, before the reconstitution with albumin, phlebitis. The response to therapy occurs often within 1-2 days, especially if commenced early in an attack. Recommendations are to complete the full 4-day course of treatment in the outpatient clinic.

Careful monitoring is advisable to detect complications early (Table 5-5). At discharge, advice should be provided for various measures to prevent further attacks (Table 5-6). Because oral contraceptives are common precipitants, gonadotropin-releasing hormone analogues can be used as alternatives given during the first few days of a cycle. Regular gynecologic assessment and bone density measurements are necessary in such cases. 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 the outcome in pregnancy.

Table 5-5 Supportive measures and monitoring in acute porphyria.

Supportive measures

- Nutritional support: oral, nasogastric, or intravenous
- Relief of pain: opiates
- Dehydration: intravenous fluids
- Insomnia and restlessness: chloral hydrate or low doses of short-acting benzodiazepines
- Nausea and vomiting: chlorpromazine and prochlorperazine
- Tachycardia and hypertension: propranolol and β -blockers but care with hypovolemia
- Seizure prophylaxis (especially if hyponatremia coexists) and control of seizures: gabapentin or vigabatrin; benzodiazepines may be safe
- Anesthesia if required: nitrous oxide, ether, halothane, and propofol
- Muscle relaxant: suxamethonium
- Bladder paresis: catheter

Monitor

- Serum electrolytes especially sodium and magnesium
- Kidney and liver tests
- Vital capacity: consider intensive care management if deteriorating
- Neurological status
- Bladder distension

Table 5-6 General and follow-up measures to be discussed with patients with acute porphyria.

Counsel
<ul style="list-style-type: none">• Avoidance of alcohol• Stopping smoking• Information about drugs safe and not safe in porphyria• Avoidance of oral contraceptives• Maintain adequate nutrition• Arrange for medical bracelets• Psychological input for depression and suicide risk• Genetic counseling for families• Photoprotection*• Avoidance of sunlight exposure and easy skin trauma*
Follow-up
<ul style="list-style-type: none">• For liver problems, especially chronic liver failure and hepatocellular carcinoma• Those with chronic hypertension needs close follow-up• Chronic pain management• Chronic mental health issues management

*For porphyrias with cutaneous manifestations only.

About 10% of patients with acute porphyria have recurrent attacks. Once weekly hematin infusions have been suggested to be useful. Repeated infusions, however, may cause venous occlusion, necessitating central venous access and iron overload because of their high iron content.

Allogeneic liver transplantation has been performed in AIP and VP patients with success. Posttransplantation, elevated urinary ALA and PBG levels returned to normal in 24 hours. Liver transplantation is dangerous, however, and should be considered only in those who experience recurrent and severe attacks. Gene therapy (adenovirus-associated virus vector delivering the PBGD gene) and enzyme replacement by recombinant human PBGD also have been attempted.

Cutaneous porphyrias

These differ from acute porphyrias mainly in the absence of neurological symptoms.

Porphyria cutanea tarda

Porphyria cutanea tarda (PCT) is the most common cutaneous porphyria and can be either sporadic (type 1) or familial (type 2). In the familial variety (20%), patients are heterozygous for uroporphyrinogen decarboxylase (UROD) mutations. The availability of half of the enzyme activity means many patients are asymptomatic unless other environmental and precipitating factors intervene. In the sporadic form (80%), in the absence of any mutations, clinical

symptoms develop when the enzyme activity decreases to <20% of normal. Recently, uroporphomethene was identified to be an inhibitor of UROD. Formation of this compound depends on hepatic iron, emphasizing the importance of iron overload as a causative factor in this condition. In addition, chronic hepatitis C and HIV, and mutations in the hemochromatosis HFE-gene (in 20% of cases), can contribute to pathophysiology of this disease by increasing liver iron. It is of value to screen the patient and first-degree relatives of patients with PCT for hereditary hemochromatosis.

PCT usually presents in adults and is characterized by bullous lesions, which starts often as erythema and becomes confluent to form the blisters. They most often are observed on the backs of the hands and other light-exposed areas. When these blisters rupture, they can cause scarring. Small white papules termed 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, a condition termed *pseudoscleroderma*. Skin symptoms show seasonal variations, with more symptoms in the summer and autumn. Rare ocular complications have been reported in PCT. Liver dysfunction is common in PCT, especially with alcohol excess and can vary from mild impairment to cirrhosis. The incidence of hepatocellular carcinoma is also higher in this cohort of patients as with acute porphyrias.

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 like alcohol, and iron supplements, phlebotomy to reduce hepatic iron is the cornerstone of treatment for this condition. Because iron overload is not marked, target ferritin can be achieved with only few phlebotomies. The plasma porphyrin level can be followed as phlebotomies are undertaken with expected control of skin lesions when elevations of the plasma porphyrins are no longer detected. Iron chelation therapy may be considered if 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 alternative to phlebotomy. Caution should be exercised in those with severe liver impairment because it may cause hepatitis. Any underlying disease like hepatitis C should be treated while opaque sun-creams containing zinc oxide should be used as sun-blocking agents.

Erythropoietic protoporphyria

Erythropoietic protoporphyria (EP), the most common porphyria in children, results from mutations in the

ferrochelatase gene and is inherited usually in autosomal-dominant fashion. In EP, skin lesions begin in early childhood. A characteristic symptom is a burning sensation developing very quickly in areas exposed to the sun. 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 myelodysplastic syndromes. Another unusual feature of this condition is the development of gallstones, in the absence of hemolysis, but this probably is due to excess amounts of protoporphyrin decreasing bile flow. Liver disease is common but typically develops after age 30. The diagnosis of the condition is made by measuring total and fractionated porphyrins and protoporphyrins. Close coordination with the reference laboratory being used is important.

Management of EP mainly includes protection from sunlight using special clothes, opaque topical sunscreens, or ultraviolet-B phototherapy. Oral β -carotene (75–200 mg/day) may help in solar sensitivity by working as an antioxidant, but it can cause a yellowish skin discoloration due to carotenemia. Melanocyte-stimulating hormone analogue, which darkens the skin, also has been tried. Because biochemical signs of iron deficiency and low vitamin D levels are frequent findings in this disease, replacement of the vitamin 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 tried, its success is limited because of continued bone marrow production of protoporphyrin. Sequential liver and bone marrow transplantation also has been described. During surgery, modification of surgical lighting is necessary to reduce injury to organs.

An X-linked form of EP due to gain-of-function mutations in the *ALAS2* gene recently has 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. Previously described mutations in *ALAS2* gene are loss-of-function ones, which causes X-linked sideroblastic anemia.

Congenital erythropoietic porphyria

Congenital erythropoietic porphyria (CEP; Günther disease), the first-described porphyria, is unique among cutaneous porphyrias in being an autosomal-recessive disorder due to deficient activity of uroporphyrinogen III synthase. The severe cutaneous photosensitivity in this case begins at birth or in early infancy in most cases. In addition to

blistering, the skin is extremely friable and easily becomes infected. Repeated infections, hypertrichosis, and bone resorption in this condition lead to severe disfigurement. Because of the deposition of excess porphyrins in the teeth, they become reddish brown (erythrodontia) and fluorescent in ultraviolet light. Corneal scarring and keratitis cause ocular problems. The excessive increase in red cell protoporphyrins (may be seen as needle-like inclusions on blood smear examination) can cause nonimmune hemolysis and subsequent splenomegaly. In certain cases, this may start in utero and manifest as hydrops fetalis.

Early diagnosis is necessary to avoid phototherapy prescription for neonatal jaundice. Red fluorescent urine in diapers is suggestive. The management strategy of CEP is based on suppressing excess erythropoiesis. In addition, sunlight protection and avoidance of skin trauma are important. Bone marrow transplantation has proved effective, whereas splenectomy and hypertransfusion have shown no benefit.

Hepatoerythropoietic porphyria

This rare condition is caused by homozygous or compound heterozygous deficiency of uroporphyrinogen decarboxylase. It tends to present in infancy or childhood and has clinical characteristics similar to CEP with red urine, skin lesions, and scarring, whereas hemolytic anemia and splenomegaly also may develop. Laboratory tests are similar to PCT, however, while treatment is based on sunlight avoidance, with no response to phlebotomy.

Pseudoporphyria

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 dermatological lesions in patients with renal failure on dialysis earning it the name "bullous dermatosis of hemodialysis." Several drugs, however, have been noted to cause this condition, including naproxen, the antibiotic nalidixic acid, dapsone, amiodarone, and diuretics. It also has been noted in individuals who use tanning beds. The clinical features of pseudoporphyria are identical to those of PCT except that the legs, upper chest, or face may be involved as well. In contrast to PCT, hypertrichosis, and hyperpigmentation, usually are not seen in pseudoporphyria. Treatment involves discontinuation of the suspected drugs (or avoidance of salons) and sun protection. Alternative analgesics with less photosensitizing properties (eg, diclofenac) may be tried. Hemodialysis-associated pseudoporphyria has responded to treatment with N-acetylcysteine in some reports.

Key points

- The most common porphyrias seen by clinicians are acute intermittent porphyria, which is an acute porphyria without skin findings, and porphyria cutanea tarda, which is primarily a cutaneous disease.
- With acute attacks of porphyria, levels of the substrate PBG or rarely ALA are increased several log-fold. Mild elevations are not diagnostic of porphyria.
- It is important for clinicians to understand the reference laboratory instructions for the correct collection and handling of specimens.
- Many more patients carry the diagnosis of porphyria than actually have the disease; thus, be critical of the diagnosis. Secondary coproporphyrinuria without PBG elevations is the most common clinical state confused with acute porphyria.
- Many mutations are described in the PBG gene. Having a genetic defect alone does not equate with disease because of an extremely varied penetrance.

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Acquired underproduction anemias
T. J. Littlewood and Siobán B. Keel

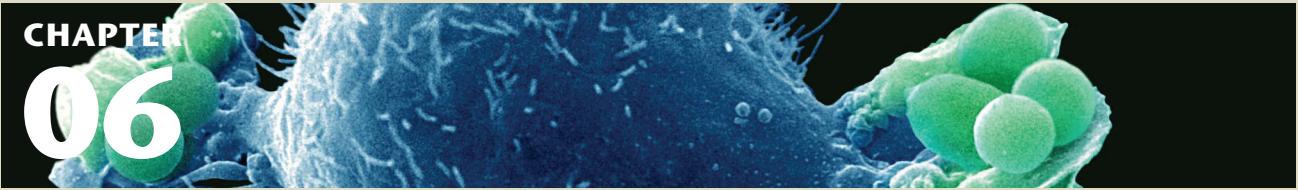
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CHAPTER
06



Acquired underproduction anemias

T. J. Littlewood and Siobán B. Keel

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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 (BFU-E), which is defined functionally by its ability *in vitro* to form large “bursts” of erythroblast colonies after approximately 2 weeks in semi-solid media. Each BFU-E generates >1,000 erythroblasts. The next defined stage is the colony-forming unit-erythroid (CFU-E), which under low concentrations of erythropoietin give rise to 8–32 well-hemoglobinized erythroblasts after approximately 1 week in culture. The erythroid stages subsequent to CFU-E are defined by their light microscopic appearance on marrow aspirate slides. Erythropoietin (Epo) is the primary cytokine that controls erythropoiesis and acts on erythroid progenitors in the stages of CFU-E to the earliest basophilic erythroblasts. Clinically, this explains why the absolute reticulocyte count increases ~7 days after an Epo signal (eg, an acute bleed) because it takes ~7 days for a CFU-E to differentiate into a reticulocyte. Epo is produced primarily in the kidney, and its mRNA expression is increased by hypoxia via the transcription factor hypoxia-inducible factor (HIF; mutations in a protein required for the destruction of HIF under normoxic conditions that result in constitutive Epo signaling, causes Chuvash polycythemia). Epo acts via cross-linkage of its receptor, resulting in activation of receptor-associated Janus kinase 2 (JAK2), which is mutated in the majority of patients with polycythemia vera (see Chapter 16). JAK2 activation initiates a sequence of signaling reactions that prevents apoptosis and stimulates proliferation and maturation of erythroid cells.

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Heme is a complex of ferrous iron and protoporphyrin IX. There are eight enzymes in the mammalian heme synthetic pathway (Figure 6-1). The first step occurs within the mitochondria where 5-aminolevulinate synthase (ALA-S2) 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 it is regulated by iron availability in erythroid cells. ALA is then transported to the cytosol where four additional enzymatic reactions occur, resulting in the production of coproporphyrinogen III, which is transported back into the mitochondria for the remaining three steps in the heme synthetic pathway, including the final step catalyzed by the enzyme ferrochelatase, where iron is incorporated into protoporphyrin IX. This is clinically relevant as ferrochelatase inserts zinc into the porphyrin ring when iron is unavailable, which can be measured in patients as erythrocyte zinc protoporphyrin (ZnPP) and may indicate iron-deficient red cell production.

In adults, approximately 200 billion erythrocytes are produced each day to replace senescent red cells that are removed from the circulation. This requires bone marrow stem cells, elemental iron, cytokines (including Epo), vitamins, and a suitable marrow microenvironment. Deficiency or unavailability of any of these key components can result 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, indicating that the marrow is not responding to the degree of anemia. The acquired and congenital (reviewed elsewhere) underproduction anemias can be further grouped by RBC size (ie, mean corpuscular volume [MCV]) into microcytic (eg, iron deficiency anemia, thalassemia), normocytic (eg, anemia of inflammation,

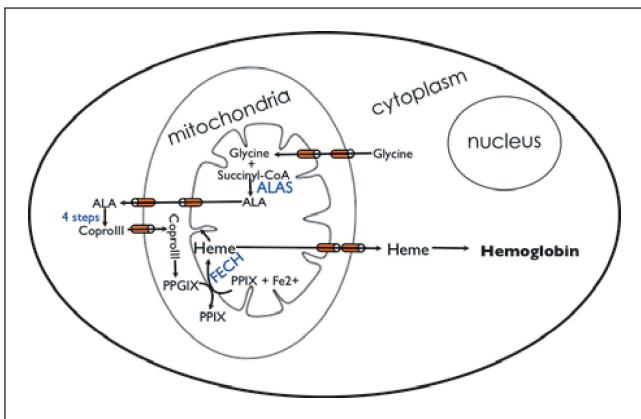


Figure 6-1 Heme synthesis. ALA = 5-aminolevulinic acid; CoproIII = coproporphyrinogen III; PPGIX = protoporphyrinogen IX; PP IX = protoporphyrin IX; ALAS = 5-aminolevulinate synthase; FECH = ferrochelatase.

anemia associated with chronic kidney disease), and macrocytic anemias (eg, megaloblastic anemias, acquired pure red cell aplasia, and myelodysplastic syndromes [MDS]). A number of other acquired anemias with low corrected reticulocyte counts are not routinely categorized by cell size (but are most often normocytic). These conditions can be complicated by multiple pathophysiologies, contributing to the suppressed RBC production, and are discussed in a separate section in 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 will focus only on the acquired (and not 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.

Microcytic anemias

Iron deficiency anemia

Iron deficiency anemia 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, mean corpuscular volume (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

Table 6-1 Select 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 B12 and folate deficiencies)

Acquired pure red cell aplasia

Anemia associated with liver disease

Acquired sideroblastic anemias (often macrocytic)[†]

Other

Anemia of cancer

Myelophthistic 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 low reticulocyte count microcytic anemias (not just those which 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.

ferritin of 9 ug/l. A workup for gastrointestinal (GI) bleeding, including upper and lower endoscopy, reveals only angiolytic lesions of the large bowel. Intravenous iron gluconate is administered with good clinical response.

Iron deficiency is the most common cause of anemia worldwide. In the United States, iron deficiency (defined by the log ratio of transferrin receptor to ferritin) is common with prevalences in children ages 1-2 and 3-5 years old of 14% and 4%, respectively. The prevalence of iron deficiency in women ages 12-19 and 20-49 years old is 9% for both age-groups (National Health and Nutrition Examination Study [NHANES] data 2003-2006).

Consideration of total iron body content and trafficking (shown in Figure 6-2) is helpful when calculating the amount of iron needed to correct a patient's iron deficit. The vast majority of the body's iron is contained in hemoglobin within erythroid cells. The iron in hemoglobin is entirely recycled. 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 ~300-600 mg in menstruating women) or other sites. Every day ~25 mg of iron recycles through this loop. Furthermore, 1-2 mg of new iron enters the body each day from dietary intake and

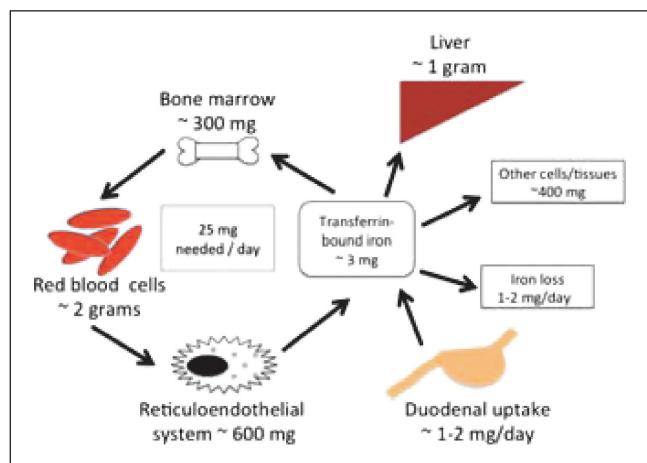


Figure 6-2 Systemic iron homeostasis. The amount of iron stored in the liver is ~1 g in men and ~300–600 mg in menstruating women.

absorption, which replaces the ~1–2 mg of iron lost daily through the sloughing of skin and intestinal cells and menstrual blood loss.

Intestinal iron absorption and the mobilization of iron from stores in macrophages and hepatocytes are controlled by both a stores- and erythroid-regulator. The stores-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 stores- and erythroid-regulators. Hepcidin acts by binding the iron export protein, ferroportin, leading to its degradation, this inhibits dietary iron absorption and macrophage iron recycling. Systemic and cellular iron homeostasis are described in detail in Chapter 5.

Intestinal iron absorption depends on the amount of iron in the diet, its bioavailability, and physiological requirements. A typical Western diet contains ~10–20 mg of iron (roughly 6 mg of iron per 1,000 calories), which exists mostly as inorganic iron (cereals and legumes) and also as heme iron (red meats, fish, poultry). Apart from legumes, most vegetables have low iron content. Inorganic iron is absorbed less readily than heme iron (in iron-replete patients, ~10% of inorganic iron vs. 30% of heme iron is absorbed). Reviewing these and other factors that affect iron absorption is useful when counseling iron-deficient patients on their dietary habits (Table 6-2).

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 6-3). 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 6-2 Factors that affect dietary iron absorption.

Inhibit absorption	Enhance absorption
Calcium-rich foods	Ascorbic acid
Tannins in tea and coffee	Heme iron
Phytates in cereals	Germination and fermentation of legumes (removes phytates)
	Ferrous iron (Fe^{2+})

In developing countries, hookworm infection leading to chronic intestinal blood loss is the most common cause of iron deficiency. In the industrial world, nonparasitic 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. Menstrual blood loss is the most common cause of iron deficiency in premenopausal females.

Several additional common causes of iron deficiency are worth noting. Approximately 5% of patients with IDA referred for evaluation by a hematologist have subclinical celiac disease, and this number appears to be higher in those patients with IDA who are unresponsive to oral iron therapy. In IDA secondary to celiac disease, abnormal iron absorption secondary to villous atrophy of the intestinal

Table 6-3 Causes of iron deficiency.

Blood loss

- Hookworm infections
- Gastrointestinal disorders (esophageal varices, hemorrhoids, peptic ulcer disease, malignancy)
- Menstruation
- Pulmonary (hemoptysis, pulmonary hemosiderosis), urologic, or nasal disorders
- Repeated blood donations or clinical blood draws or factitious blood removal
- Dialysis
- Intravascular hemolysis with hemoglobinuria (paroxysmal nocturnal hemoglobinuria, prosthetic heart valve)

Increased iron requirements

- Rapid growth during infancy and adolescence
- Erythropoietin therapy
- Pregnancy and lactation

Inadequate iron supply

- Poor dietary intake (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/*Helicobacter pylori* colonization
 - Congenital disorders of iron transport (iron-refractory iron deficiency anemia, hereditary hypotransferrinemia, divalent metal transporter 1 disease)

mucosa and a component of anemia of inflammation 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 nutritional deficiency associated with celiac disease.

Iron absorption is heavily dependent on normal gastric acid secretion for solubilizing and reducing dietary iron. Thus, iron absorption is impaired by achlorhydria. Autoimmune atrophic gastritis (defined as hypergastrinemia and strongly positive antiparietal cell antibodies) is another common cause of IDA seen by hematologists. Eradication of *Helicobacter pylori* colonization, which can coexist with autoimmune atrophic gastritis and may share a common pathophysiologic mechanism, in infected individuals with refractory IDA has been shown to result in an appropriate response to oral iron therapy and normalization of hemoglobin levels. Whether and how *H. pylori* colonization causes iron deficiency remains unknown. Celiac disease, autoimmune atrophic gastritis, and *H. pylori* infection should be considered in patients with iron deficiency and particularly in those refractory to oral iron therapy.

Another acquired cause of iron deficiency worth highlighting is iron deficiency in infants resulting from inadequate dietary iron intake. This occurs in ~20%-40% of infants who drink cows' milk or nonfortified formula as a sole source of nutrition. Several factors may act synergistically: (i) cows' milk has a low iron content, (ii) calcium and milk proteins can inhibit absorption of nonheme dietary iron, and (iii) consumption of cows' milk is associated with intestinal blood loss. Also of note, IDA is common during pregnancy (discussed in the section "Anemia associated with pregnancy").

Iron-refractory iron deficiency anemia (IRIDA) is a disease characterized by a congenital hypochromic, microcytic anemia, and low serum transferrin saturation, which is refractory to oral iron therapy and only partially responsive to parenteral iron. Although this is congenital, and not

acquired, underproduction anemia, it is included in this section to complete this review of IDA. The molecular basis of this disease was recently defined. IRIDA is caused by mutations in TMPRSS6, a transmembrane serine protease. These mutations result in inappropriately high hepcidin levels.

Stages of iron deficiency and clinical manifestations

The manifestations of iron deficiency occur in several stages (Table 6-4), 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 a decreased serum ferritin. As iron stores become exhausted, the TIBC begins to rise and serum transferrin saturation begins to fall. When erythropoiesis becomes iron restricted, erythrocyte ZnPP levels increase and the cells become microcytic. With further iron depletion, anemia develops.

Iron-deficient individuals may have no symptoms, non-specific symptoms of anemia, or other symptoms. Ice eating (pagophagia) and other forms of pica are seen in a minority of cases. Findings on physical examination become more pronounced as the iron deficiency worsens and include pallor, stomatitis, glossitis, koilonychia of the nails, and other symptoms due to the effects of iron on rapidly dividing cells. 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. Almost all have addressed women exclusively and have focused on nonscarring hair loss. Some suggest that iron deficiency may be related to various forms of hair loss, whereas others do not.

Diagnosis and treatment

In classical IDA, a patient presents with an obvious clinical history of blood loss and a low reticulocyte count, microcytic

Table 6-4 Stages of iron deficiency.

	Normal	Iron deficiency	Iron-restricted erythropoiesis	Iron deficiency anemia
Marrow iron stores	+++	None	None	None
Marrow sideroblasts	Present	Present	Absent	Absent
Ferritin (ug/l)	~40-200	~20-30	~10-15	<10
MCV	Normal	Normal	Slight microcytosis	Microcytic
Anemia	Absent	Absent	Absent	Present
TIBC (ug/dL)	Normal	Normal	Normal-mildly increased	Increased
Fe (ug/dL) depends on diet	~60-150	~<40	~<20	~<10
Transferrin saturation (%)	20-50	30	<15	<15
Erythrocyte zinc protoporphyrin (ng/mL)	~30-70	~30-70	>100	~100-200

Fe = iron; MCV = mean corpuscular volume; TIBC = total iron-binding capacity.

and hypochromic (low mean corpuscular hemoglobin, MCH) anemia, and an elevated platelet count. The mechanism of thrombocytosis in IDA is unknown. Iron studies reveal a low serum ferritin, serum iron, and transferrin saturation, and an 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. Target cells may be seen; however, this finding is difficult to interpret as it can reflect a drying artifact in smear preparation. Table 6-5 compares the classical laboratory results in iron-restricted erythropoiesis and anemia of chronic inflammation.

Unfortunately, IDA rarely presents classically and routine iron studies have limitations, which 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 ug/l serum ferritin corresponds to ~8–10 mg of tissue iron stores and is an excellent outpatient screen for iron deficiency. In women of reproductive age, a ferritin of <5 ug/l is diagnostic of iron deficiency (defined as no stainable bone marrow iron stores) with a reported specificity of 98% and a sensitivity of ~75%. A higher ferritin cutoff for suspecting iron deficiency may be appropriate in some populations; a serum ferritin of <30 ug/l in an anemic patient without inflammation was reported to be 92% sensitive and 98% specific in diagnosing IDA. Ferritin is an acute phase reactant, and its plasma level is increased in liver disease, infection, inflammation, and malignancy. IDA should be considered in these patient populations when anemia and a serum ferritin of ~60 ug/l are present. Despite its limitations, serum ferritin is superior to serum transferrin, transferrin saturation, MCV, RDW, and erythrocyte ZnPP in diagnosing iron deficiency. A serum ferritin of <30 ug/l is useful in diagnosing iron deficiency in pregnant women (sensitivity of ~90% and specificity of ~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 because of ingestion of iron, diurnal rhythm, and other factors. Transferrin is affected by nutritional status and transferrin saturation is a function of serum iron and transferrin.

A number of additional studies can support a diagnosis of IDA in situations in which serum ferritin is equivocal. Increased RDW is sensitive for diagnosing IDA, but it lacks specificity. A trend of decreasing MCV and increasing RDW over time can be instructive. Erythrocyte ZnPP levels are increased in iron deficiency as zinc, rather than iron, is incorporated into the protoporphyrin ring when iron is unavailable. ZnPP has a high sensitivity for detecting iron deficiency in adults but lacks specificity as it also is increased in lead poisoning, anemia of chronic inflammation, and some hemoglobinopathies. The percentage of circulating hypochromic erythrocytes and the reticulocyte hemoglobin concentration detect early iron-deficient erythropoiesis. They are limited, however, by false normal values seen in patients with elevated MCVs or with thalassemia, and the requirement for a specialized analyzer not available in most laboratories, respectively. Measurement of serum-soluble transferrin receptor (sTfR) also may be used to estimate body iron stores. The level of sTfR is directly proportional to the total amount of cell surface-associated transferrin receptor 1 (TfR1) and thus reflects both the quantity of erythroid precursors and the cell surface expression of TfR1 per cell. Levels are increased in iron deficiency and anemias with ineffective erythropoiesis (eg, thalassemia, MDS, folate deficiency, and vitamin B12 deficiency) in which there is an imbalance between the amount of iron that is endocytosed by marrow erythroblasts and the amount of iron incorporated into circulating erythrocytes. The incorporation of sTfR into the sTfR-ferritin index (sTfR/log₁₀ferritin) has shown more promise in distinguishing IDA from anemia of

Table 6-5 Iron studies in IDA versus anemia of chronic inflammation.

	IDA	Anemia of chronic inflammation
Serum ferritin	Decreased	Normal or decreased
Serum iron	Normal or decreased	Normal or decreased
Total iron binding capacity or transferrin	Increased	Normal or decreased
% iron saturation	Decreased (<10%-15%)	Normal or decreased
MCV	Decreased	Normal or decreased
RDW	Increased	
sTfR/log ferritin ratio	>2	<1
Hepcidin (not currently clinically available)	Suppressed	Increased

IDA = iron deficiency anemia; MCV = mean corpuscular volume; RDW = red blood cell distribution width; sTfR = serum-soluble transferrin receptor.

chronic inflammation than sTfR1 alone. While commercially available, sTfR testing lacks standardization and is not widely available.

Serum hepcidin, the primary regulator of iron homeostasis in the body, is suppressed in iron deficiency. The utility of measuring serum hepcidin in the workup of iron deficiency and other disorders of iron homeostasis remains undefined and warrants further investigation before being accepted into widespread clinical practice.

Evaluation of the bone marrow for stainable iron had been considered the gold standard for the diagnosis of iron deficiency. High interobserver variability and the expense and invasiveness of the test limit its clinical utility, however. A trial of oral iron therapy with close laboratory and clinical follow-up is simultaneously diagnostic and therapeutic and has a role in specific clinical scenarios.

Once iron deficiency or IDA is confirmed, a search for the underlying etiology (Table 6-3) should be initiated. Upper and lower GI endoscopies should be pursued in all males and postmenopausal female patients with confirmed IDA. If menstrual blood loss is significant and appears to be the primary source of IDA, then a trial of iron therapy is reasonable before proceeding to GI evaluation in carefully selected premenopausal female patients who are followed closely for response to therapy. Some experts advocate serological and biochemical screening when there is a clinical suspicion of celiac disease (antiendomesial and antitransglutaminase antibodies) and atrophic gastritis (gastrin and antiparietal cell antibodies) or in otherwise-unexplained IDA patients; others advocate universally screening for celiac disease in all IDA patients. Cases of suspected celiac disease should be confirmed by duodenal biopsy. If IDA persists or recurs after a normal esophagogastroduodenoscopy (EGD) and colonoscopy, it is reasonable to seek *H. pylori* by noninvasive testing and eradicate it, if present. 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 EGD and colonoscopy). An oral iron absorption test is 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 90 minutes after administration of ferrous sulfate (5 mg/kg). In a patient with IDA with normal intestinal iron absorption, the serum iron level is expected to increase by at least 50 ug/dL 90 minutes after the oral iron challenge. The test is less readily interpretable in a nonfasting patient.

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 is available on numerous pharmacy websites). This quantity should be considered in the context of intestinal iron absorption when considering the likelihood of replacing the deficit by oral administration (constipation secondary to oral iron replacement can be dose limiting in some patients) or to define the amount of parenteral iron to administer.

Oral iron salts are a safe first-line treatment for iron deficiency. There is no evidence to suggest that a particular oral iron preparation is more effective or better tolerated than another in equivalent doses. The typical replacement dose of elemental iron in adults is 100-200 mg/day and 3-6 mg/kg/day in infants and children (doses split into divided doses). Ferrous sulfate is available in 325 mg tablets (each containing 65 mg of elemental iron) and ferrous gluconate is available in 320 mg tablets (each containing 32 mg of elemental iron). Ferrous sulfate elixir (a liquid formulation) is available for infants and young children. Nausea, vomiting, epigastric discomfort, and constipation are common dose-dependent side effects of iron salts; ~25% of patients cannot tolerate oral iron because of side effects. Patients should be alerted that iron might turn their stools a darker color. 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. Antacids, the tannins found in tea, calcium supplementation, bran, and whole grains, if taken concurrently with oral iron, can all decrease iron absorption. Treatment with oral iron should continue for ~3 months after the hemoglobin normalizes to replete iron stores.

Oral iron supplementation is the preferred replacement route in most uncomplicated cases of iron deficiency. Parenteral iron should be given intravenously (intramuscular is painful) and is indicated when there is an absolute noncompliance with or intolerance to oral iron therapy, high iron requirements, or proven intestinal malabsorption. 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/million) and thus no longer should be used. Low-molecular-weight iron dextran is considerably safer than its high-molecular-weight counterpart. The advantages of low-molecular-weight iron dextran include its low cost and the ability to give replacement doses of iron in a single infusion. Iron sucrose and iron gluconate have very low incidences of anaphylaxis (no fatal cases of

anaphylaxis have been reported), and their administration does not require a test dose. Side effects of iron sucrose and iron gluconate include mild arthralgias and myalgias. Disadvantages of these newer formulations include greater cost and the inability to give a total replacement dose in a single infusion because they cause GI and vasoactive reactions at doses >200–400 mg. Newer iron preparations have been developed to enable more rapid high-dose bolus injections. Ferumoxytol enables a bolus injection of 510 mg to be administered in 17 seconds. Ferric carboxymaltose is licensed in Europe, and it enables 1,000 mg of iron to be given as an intravenous infusion over 15 minutes.

IDA patients receiving supplemental iron generally respond with a reticulocytosis within 7–10 days of initiating treatment. A 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 replete body stores. The failure to respond to oral iron should prompt consideration of ongoing bleeding, poor patient compliance, poor iron absorption, inadequate replacement dosing, or appropriateness of the diagnosis.

Key points

- Iron deficiency is the most common cause of anemia worldwide and its diagnosis requires a search for the underlying etiology.
- Classical iron deficiency is characterized by a low corrected reticulocyte count, hypochromic, microcytic anemia, and elevated RDW.
- A ferritin of <15 ug/l in a premenopausal woman is diagnostic of iron deficiency.
- Oral iron supplementation is the preferred replacement route in most uncomplicated cases of iron deficiency.
- IDA is the most common nutritional deficiency associated with celiac disease.
- Failure to respond to oral iron should prompt consideration of ongoing bleeding, poor patient compliance, poor iron absorption, inadequate replacement dosing, or appropriateness of the diagnosis.

Normocytic anemias

Anemia of chronic inflammation (anemia of chronic disease)

Anemia of chronic inflammation 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 a decreased serum iron, low TIBC, and low percent iron saturation. Serum ferritin is 55 ng/mL, and the erythrocyte sedimentation rate (ESR) is elevated 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.

Anemia is common in patients with chronic inflammatory conditions, such as malignancy, autoimmune diseases, chronic infections, and chronic kidney disease. The resulting anemia is termed the anemia of chronic inflammation or the anemia of chronic disease (AOCD). It is now recognized that patients with illnesses not traditionally thought to be inflammatory in origin, such as trauma, postsurgery, and prolonged critical illness, also may develop AOCD. AOCD is generally a sign of underlying disease activity and a thorough search for an underlying disorder should be performed when diagnosing AOCD as the cause of anemia. Patients with AOCD typically have hemoglobin levels in the range of 7 to 11 g/dL. The anemia in AOCD is characterized as a low corrected reticulocyte count, normochromic, and normocytic anemia. Over time, however, the anemia may become more severe with microcytic and hypochromic indices. Although laboratory values overlap and may not be discriminating, iron studies often are used to differentiate AOCD from IDA. Typically, iron levels are low to normal, iron-binding capacity is low to low normal, and ferritin is normal or elevated in AOCD (Table 6-5). In many but not all conditions, an elevated ESR or C-reactive protein (CRP) may be a clue to the diagnosis of AOCD. Multiple processes are involved in the pathogenesis of AOCD. Cytokines, such as tumor necrosis factor α ($TNF\alpha$), interleukin 1 (IL-1), interleukin 6 (IL-6), and interferons play a central role in the abnormalities observed in these patients. These cytokines cause a reduction in the proliferation of erythroid precursors in response to erythropoietin, a decrease in the production of erythropoietin 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, cause an increase in the hepatic synthesis of hepcidin, the key regulator of iron transmembrane transport. Hepcidin acts by binding the iron export protein, ferroportin, leading to its degradation; this inhibits dietary iron absorption and macrophage iron recycling. This is reflected in low plasma iron levels and results in iron-restricted erythropoiesis.

In infants and children, inflammatory anemia does not require the presence of an underlying chronic inflammatory disorder. Even minor bacterial or viral infections, when

recurrent, can cause a mild normocytic anemia with blunted reticulocyte response within a few weeks. Their pathophysiologies likely mirror AOCD. This form of anemia inflammation is self-limited and resolves when the infant is free of infection.

In most patients with AOCD, the anemia is mild and improves with the treatment of the underlying disorder. Patients may have concomitant true iron deficiency or functional iron deficiency. If treatment becomes necessary, erythropoiesis stimulating agents (ESAs) have been shown to be beneficial in some patients +/– supplemental iron. The most experience in using ESAs and supplemental iron to correct an acquired underproduction anemia is in patients with chronic kidney disease. AOCD is one pathophysiology contributing to their 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 low corrected reticulocyte count, normocytic, or microcytic anemia with decreased serum iron, a decreased concentration of transferrin (or TIBC), and a decreased transferrin saturation with normal or increased serum ferritin.
- The pathophysiology of AOCD is multifactorial, but the sequestration of iron secondary to elevated hepcidin levels plays a central role.
- Treatment should be directed at the underlying medical condition.

Anemia associated with chronic kidney disease

Anemia associated with chronic kidney disease 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 9.7g/dL and an MCV of 77 fl. Iron studies are consistent with IDA. Intravenous iron dextran is administered with good clinical response.

The anemia associated with chronic kidney disease is primarily due to an underproduction of erythropoietin secondary to a decrease in the number of renal cortical cells available to produce the hormone. The accumulation of uremic toxins also may cause reduced synthesis of erythropoietin. The ability of the kidney to secrete erythropoietin deteriorates as renal function worsens; however, a direct correlation to the degree of renal dysfunction varies greatly between patients.

For example, patients with diabetes often have anemia out of proportion to the apparent reduction in renal function. The anemia in many patients with chronic kidney disease also has features of the AOCD.

Other contributing causes to the anemia include blood due to uremic platelet dysfunction, in the dialysis circuit, or from frequent phlebotomies and bleeding GI angiodysplasias (common in uremic patients). These losses may amount to up to a 2-g loss of iron annually. Secondary hyperparathyroidism may contribute to the anemia through suppression of the bone marrow. Erythrocyte survival may be mildly to moderately decreased in a subset of patients with renal insufficiency. In the setting of uremia, RBCs become less deformable and are more susceptible to mechanical destruction and clearance by macrophages. Metabolically, uremia causes the erythrocyte to have decreased activity of enzymes involved in the hexose-monophosphate shunt and decreased ATPase activity. This leads to increased susceptibility to oxidative stress and abnormal membrane permeability, which in turn can lead to decreased erythrocyte life span. Hemodialysis may introduce RBC toxins, such as copper, formaldehyde, chlorine, nitrates, and chloramines, which can damage RBCs and decrease survival. The mechanical process of dialysis can lead to RBC fragmentation.

Anemia associated with chronic kidney disease is usually normochromic and normocytic unless complicated by iron deficiency or vitamin deficiencies. The reticulocyte count is low. The peripheral smear is often normal, but in patients with severe kidney failure, it may show erythrocytes with short cytoplasmic extensions termed burr cells or echinocytes (Figure 6-3).

Burr cells

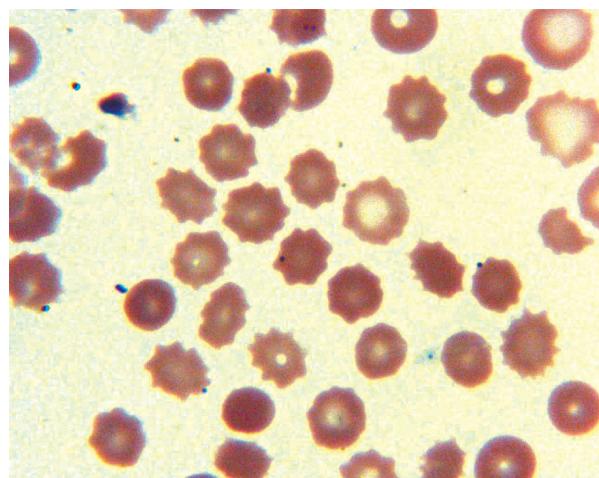


Figure 6-3 The small, blunted projections of the burr cells circumscribing the entire red cell membrane are readily apparent in this view. This morphologic feature should not be confused with spur cells (acanthocytes), which have spiculated membrane projections of variable size at random points on the red cell membrane.

The bone marrow usually 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 but may show low serum iron levels, accompanied by low serum transferrin levels and high ferritin, as seen in AOCD. Because of the multifactorial nature of the anemia in renal disease, there is a wide range of possible hemoglobin concentrations for any given degree of renal dysfunction (Figure 6-4). Studies have demonstrated that the prevalence of anemia is greater in persons with creatinine clearances <60 mL/min/1.73 m²; however, this population-based trend cannot be applied to individual patients. Anemia may be present at higher levels of creatinine clearance, underscoring the importance of measuring hemoglobin in people with renal disease and the need for individualized assessment of anemia.

The treatment of anemia in patients with chronic kidney disease has been transformed by the development and use of recombinant human erythropoietin and other ESAs. Recent studies have shown that targeting normal or near-normal hemoglobin values is associated with increased risk of adverse cardiovascular events and mortality compared with lower target hemoglobin levels. The mechanism to account

for these clinical outcomes remains poorly understood. The 2007 National Kidney Foundation Dialysis Outcomes Quality Initiative (DOQI) Guidelines for the anemia of chronic kidney disease recommend a target hemoglobin levels in the range of 11-12 g/dL in all patients with chronic kidney disease.

Approximately 25% of dialysis patients have a relative resistance to ESA. Common causes of ESA resistance or refractoriness include infection, inflammation, functional or true iron deficiency, bleeding, and hemolysis. Functional iron deficiency occurs when iron is present in body stores (liver), but its delivery to developing RBCs is blocked by the impact of hepcidin. Functional iron deficiency is suspected when the ferritin is <100 ug/l and the transferrin saturation is $<20\%$. Nephrologists routinely supplement patients with intravenous iron to maintain a serum transferrin saturation $>20\%$ and a serum ferritin >100 ug/l. Antierythropoietin antibodies resulting in pure red cell aplasia are a rare cause of ESA resistance. There was an epidemic of such cases about 12 years ago caused by changes in the manufacturing process for epoetin- α . Most of the affected patients were treated in Europe and received subcutaneous rather than intravenous injections of the ESA.

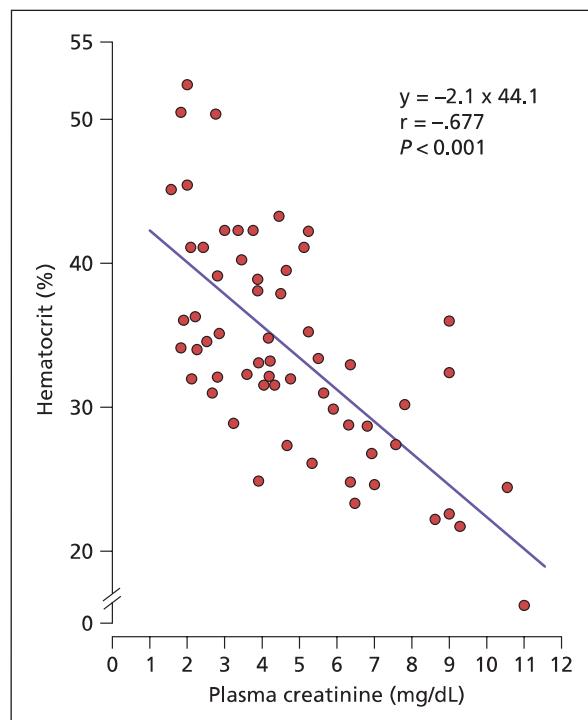


Figure 6-4 Relationship between hematocrit and plasma creatinine concentration in 60 patients with varying degrees of renal insufficiency. The severity of anemia is directly related to the level of reduced renal function. Reprinted with permission from McGonigle RJS, Wallin JD, Shadduck RK, et al. Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. *Kidney Int*. 1984;25:437-444.

Key points

- Renal disease is the prototypical disorder of erythropoietin deficiency.
- ESAs are effective treatment of anemia associated with chronic kidney disease.
- Causes of a failure to respond to an ESA in patients with kidney disease include either true or functional iron deficiency, folic acid deficiency, coexistent infection or inflammatory disease, or ongoing bleeding or hemolysis.

Macrocytic anemias

Megaloblastic anemias

Megaloblastic anemias are characterized by a low corrected reticulocyte count, a marked macrocytosis (often macroovalocytes), hypersegmented neutrophils, megaloblastic changes in marrow morphology, and occasionally pancytopenia. These findings all reflect impaired DNA synthesis in hematopoietic cells. Megaloblastic morphologic changes in the marrow resulting from the dyssynchrony between nuclear and cytoplasmic maturation may include the presence of giant pronormoblasts and giant metamyelocytes. RBC development is ineffective as defined by an imbalance between the amount of iron that is endocytosed by marrow erythroblasts and the amount of iron incorporated into circulating erythrocytes, implying death of cells within the marrow. This is clinically reflected by an elevated lactic

dehydrogenase (LDH) and unconjugated bilirubin and low haptoglobin. Cobalamin and folate deficiency are the most common causes of megaloblastic anemias.

Vitamin B12 deficiency

Cobalamin deficiency 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 by transurethral resection and an intravesical Bacillus Calmette-Guerin (BCG) vaccine. He has type 2 diabetes treated with metformin. On examination he is pale, has mild peripheral edema and minimal loss of position, and has vibratory sense in the feet bilaterally. Laboratory evaluation reveals a hemoglobin of 7.1 g/dL, MCV of 135 fl, neutrophil count of 960/mL, and platelet count of 35,000/ μ L. A 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.

Vitamin B12 (cobalamin) functions as an essential coenzyme for two enzymes in the human body: cytoplasmic methionine synthase, which catalyzes methylation of homocysteine to methionine; and methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA in the mitochondria. The succinyl-CoA formed in the latter reaction enters the Krebs cycle. The former reaction 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.

Humans are completely dependent on a dietary source of cobalamin, namely meat products. In the upper GI tract, cobalamin is released from food components and is bound to haptocorrin, a protein present in saliva and gastric fluids, which likely protects the vitamin from degradation in the acidic stomach. In the duodenum, pancreatic enzymes degrade haptocorrin, and cobalamin subsequently binds to intrinsic factor. Intrinsic factor is synthesized and secreted by the parietal cells of the stomach. In the terminal ileum, intrinsic factor-bound cobalamin is endocytosed by the receptor complex, cubam. Inside the ileal enterocyte, the intrinsic factor is degraded and the released cobalamin exits the basolateral surface of the cell 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 in rare cases, from insufficient dietary intake or defects in bodily transport. Select causes of cobalamin deficiency are listed in Table 6-6. Owing to efficient enterohepatic circulation, as well as reuptake in

the kidney, cobalamin is retained in the body for long periods, and thus dietary deficiency requires years to develop.

The most common cause of symptomatic cobalamin deficiency is pernicious anemia (PA). PA is a macrocytic anemia due to cobalamin deficiency that results from atrophy of the stomach's mucosa and impaired secretion of intrinsic factor. The disease generally presents in patients >50 years old. It is considered an autoimmune disorder because of the frequent presence of autoantibodies directed against the intrinsic factor as well as against parietal cells, and its association with other autoimmune disorders (eg, thyroid disease, type 1 diabetes, and vitiligo). The presence of antibodies to intrinsic factor are pathognomonic of PA, but they are present in only ~70% of cases. Antiparietal cell antibodies also may be present. PA often is considered a synonym of autoimmune gastritis because PA is thought to be the end stage of an autoimmune process that results in severe damage to the gastric mucosa.

Experimental and clinical data strongly suggest an involvement of long-standing *H. pylori* infection in the pathogenesis of PA and atrophic body gastritis. Atrophic body gastritis is characterized by a severe diffuse atrophy of the oxyntic glands and hypochlorhydria. Of note, malabsorption of cobalamin because of hypochlorhydria (whether or not a consequence of atrophic body gastritis) is probably a common explanation for subnormal vitamin B12 concentrations in elderly patients. PA and atrophic body gastritis may be examples of pathogen-induced, organ-specific autoimmunity that

Table 6-6 Select causes of B12 deficiency.

Impaired absorption

- Hypochlorhydria (impairs release of B12 from dietary proteins)
- Age
- Gastric atrophy (*Helicobacter pylori* or autoimmune gastritis)
- Medications (proton-pump inhibitors or H₂ antagonists)
- Inadequate pancreatic protease (B12 remains sequestered by haptocorrin)
- Intestinal competition for host B12 (tapeworm *Diphyllobothrium latum*)
- Deficiency of intrinsic factor or IF-bound B12 uptake
 - Pernicious anemia
 - Congenital intrinsic factor deficiency
 - Gastric bypass surgery
- Decreased ileal absorption of B12
- Ileal resection or bypass
- Ileal dysfunction (Crohn's disease, Celiac disease, intestinal lymphoma, bacterial overgrowth from blind loop syndrome)
- Medications (Metformin, mechanism unknown)

Insufficient dietary intake

(strict vegans, rarely some vegetarians, and in some developing nations)

Defects in bodily transport

- Congenital disorders of vitamin B12 transport (defects in cubam, transcobalamin, others)

develops in genetically susceptible patients through a loss of immunological tolerance. The active infectious process is replaced gradually by an autoimmune process mediated by autoreactive gastric T-cells that recognize H⁺/K⁺-ATPase and *H. pylori* antigens, which culminates in a burned-out infection and irreversible damage to the gastric body mucosa.

PA patients are at risk for the development of gastric adenocarcinoma and carcinoid tumors. Endoscopy at presentation is commonly done; however, data are insufficient to support routine subsequent endoscopic surveillance of these patients. Follow-up should be individualized to the patient.

Diagnosis and treatment

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 paresthesias, unsteady gait, or clumsiness, and are caused by lesions in the posterior and lateral columns of the spinal cord (subacute combined degeneration) and in the cerebellum. It is critical to recognize these symptoms early to avoid irreversible neurologic dysfunction. Folate replacement alone may improve the anemia in patients with cobalamin deficiency, thereby masking the underlying cobalamin deficiency and allowing progression of the neurologic deficit. Therefore, cobalamin levels always should be measured before initiation of folate in patients at risk for concomitant cobalamin deficiency.

No diagnostic gold standard exists for diagnosing cobalamin deficiency and each laboratory test has some disadvantages. Low serum cobalamin levels (<200 pg/mL, ~97% sensitive) are diagnostic of deficiency and levels >300 pg/mL speak against deficiency. Methylmalonic acid (MMA) and total homocysteine (HCY) generally are considered to be more sensitive indicators of early deficiency; serum levels of both MMA and HCY become elevated before cobalamin levels fall below the lower limits of the normal range (200-250 pg/mL). Measuring MMA (\pm HCY) levels may be useful in patients with symptoms consistent with cobalamin deficiency and in whom serum cobalamin levels are equivocal (200-300 pg/mL), patients with atypical clinical findings in whom cobalamin deficiency must be ruled out, and asymptomatic patient accidentally 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. Recently, assays to measure cobalamin bound to transcobalamin, and thus cobalamin available for delivery to tissues (holoTC), became available. How much additional diagnostic value holoTC adds to current studies remains unclear and assay availability currently is limited.

There is debate on the clinical importance of identifying patients without overt cobalamin deficiency symptoms who have laboratory tests suggesting cobalamin deficiency, so-called subclinical cobalamin deficiency. Subclinical deficiency, as defined by an elevated MMA and normal cobalamin level, is present in ~15% of elderly patients. The majority of patients with subclinical cobalamin deficiency do not progress to symptomatic cobalamin deficiency. It remains uncertain whether these patients may have subtle and clinically unrecognized symptoms of cobalamin deficiency and controversial whether they should be treated or followed closely. Currently, routine screening of asymptomatic individuals for cobalamin deficiency is not recommended.

Low cobalamin levels alone (without overt megaloblastic anemia or typical neurological symptoms) also can be seen in association with a variety of other clinical conditions including pregnancy, because of 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), of MMA alone (intestinal overgrowth), or of both (renal failure). Whether elevated HCY levels associated with cobalamin deficiency increase a patient's risk of vascular thrombosis is controversial. Of note, 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.

Reasonable testing for cobalamin deficiency includes measuring both a serum cobalamin level and at least one of the other metabolic tests (possibly MMA). Serum cobalamin testing alone is adequate to establish a diagnosis of cobalamin deficiency if the level is <200 pg/mL and other clinical signs and symptoms support the diagnosis. Once cobalamin deficiency is confirmed, patients should be evaluated for the underlying cause. Because PA is the most common etiology, antibodies against intrinsic factor (50%-70% sensitive, ~100% specific) should be assessed. Antiparietal cell antibodies support a diagnosis of PA but lack specificity. Measuring both anti-intrinsic factor and antiparietal cell antibodies appears to improve the diagnostic yield. Histologic confirmation of gastric body atrophy is required for the diagnosis. Historically, the Schilling test was used to measure cobalamin absorption, but this test is no longer available in most centers.

Cobalamin deficiency in infants and children is uncommon. Rare cases of cobalamin deficiency due to a congenital defect in intrinsic factor secretion from parietal cells (ie, congenital PA) present around 18-36 months of age, after the depletion of fetal liver stores. Typical acquired PA also may

present in children. 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 explained by the additional function of cubam 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 normal intrinsic factor secretion, cobalamin absorption, and cobalamin levels.

Patients with cobalamin deficiency can be treated with parenteral or oral cobalamin replacement. Parenteral therapy is recommended for patients with significant symptomatology. Cyanocobalamin is the only form available in the United States. Intramuscular cyanocobalamin is given in doses of 1,000 ug/day for 1 week, and then 1,000 ug/week for 4 weeks, and if an underlying disorder persists, 1,000 ug/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. Oral cobalamin may be a safe and effective treatment in some patients, even when intrinsic factor is present at low levels. The initial oral replacement dose begins at 1,000-2,000 ug/day. Patients treated with oral cobalamin should be observed carefully to ensure that symptoms of anemia improve. After cobalamin replacement is commenced, some patients will become hypokalemic or iron deficient because of potassium and iron uptake by developing erythroid cells. This requires replacement.

Following cobalamin replacement, the marrow shows resolution of megaloblastic changes within hours and reticulocytes appear in the peripheral blood, usually peaking approximately 1 week after starting replacement therapy. Hypersegmentation of neutrophils may persist for up to 2 weeks. Blood counts and MCV return to normal in 2-3 months. Neurologic abnormalities usually resolve within 3 months, although in some patients, this may take as long as 6-12 months. In some individuals the neurological deficits are irreversible.

Key points

- The most common cause of cobalamin deficiency is impaired absorption.
- PA is the most common cause of impaired cobalamin absorption, resulting in symptomatic deficiency.
- Both cobalamin and folate deficiency cause a megaloblastic anemia; however, neuropsychiatric symptoms are seen only in cobalamin deficiency.
- Parenteral therapy is recommended for patients with any neuropsychiatric symptoms.
- Subclinical cobalamin deficiency (defined by elevated MMA and HCY levels with no clinical signs or symptoms) is of uncertain significance.

Folic acid deficiency

Folate acid deficiency 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, 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/mL, and platelet count of 55,000/mL. A serum folate level is 1 ng/mL, cobalamin level is 350 pg/mL. He is enrolled in an alcoholic treatment program and started on 2 mg of daily oral folic acid replacement with symptomatic improvement and brisk reticulocytosis noted within 2 weeks.

Folate exists in nature as a conjugate with glutamic acid residues. Folate is important biologically as a transporter of single carbon fragments, but to do so it must be reduced to tetrahydrofolate. It is used in the synthesis of both purines and pyrimidines and participates in the conversion of uracil to thymidine. Folates are maintained intracellularly by conjugation with glutamic acid residues. Folate must be deconjugated to contain only one glutamic acid residue to be absorbed from the duodenum and proximal jejunum. Once it is in its mono-glutamate form, cells can take up folate. Recently, a proton-coupled high-affinity folate transport protein expressed in the duodenum and jejunum (PCFT/HCP1) was identified. A loss-of-function mutation in the gene encoding PCFT/HCP1 results in a syndrome of hereditary folate malabsorption.

Folate deficiency may result from decreased dietary intake, impaired absorption, or increased utilization (Table 6-7). The major cause of folate deficiency is decreased dietary intake rather than impaired absorption, which is the major cause of cobalamin deficiency. Green leafy vegetables (eg, spinach and turnip greens), fruits (eg, citrus fruits and juices), and dried beans and peas are all natural sources of folate. The implementation of folic acid fortification of grains in many countries has drastically reduced the prevalence of folate deficiencies in those countries. The FDA-recommended daily dietary folate equivalent is 400 ug. Folate deficiency due to inadequate dietary intake can develop in months since body stores are not extensive. Folate supplementation should be part of routine prenatal care to reduce the risks of neural tube defects. Folate supplementation should be considered in other patients with increased folate requirements (eg, chronic hemolytic anemia patients).

Diagnosis and treatment

The hematologic manifestations of folate deficiency are indistinguishable from cobalamin deficiency. Folate deficiency does not cause subacute combined degeneration. Folate deficiency is strongly implicated in increasing the incidence of neural tube defects in the fetus. Plasma (or serum) folate undergoes diurnal

Table 6-7 Select causes of folate deficiency.

Impaired absorption
Decreased duodenal and ileal absorption of folate
Intestinal dysfunction (Crohn's disease, Celiac disease)
Congenital abnormality in intestinal folate transporter (mutations in <i>PCFT</i>)
Insufficient dietary intake
Poor nutrition
Old age ("tea and toast" diet)
Alcoholism
Prolonged hyperalimentation
Developing countries that lack folate fortified foods
Increased requirements
Increased cellular proliferation
Pregnancy
Lactation
Hemolytic anemia (sickle cell anemia, warm autoimmune hemolytic anemia)
Malignancies (associated with a high proliferative rate)
Exfoliative dermatitis
Hemodialysis
Medication that affect folate metabolism or possibly absorption (methotrexate, phenytoin, carbamazepine)

changes related to recent food intake, which limits the diagnostic usefulness of the assay. If the serum folate is >4 ng/mL, folate deficiency is unlikely. If the serum folate concentration is <2 ng/mL, folate deficiency is likely. Alternatively, RBC folate levels remain constant from day to day and accurately reflect the average folate content of the long-lived circulating RBC population. However, care must be taken when interpreting this laboratory measurement, as RBC folate levels may also be low in people with cobalamin deficiency, and additionally, the RBC folate assay methodology is problematic. Folate deficiency also results in high levels of HCY (but not MMA). Folate-deficient people are treated with folic acid (1-5 mg/day) for 1-4 months, or until complete hematologic recovery occurs. Folic acid can partially reverse the hematologic abnormalities of cobalamin deficiency while the neurologic symptoms resulting from cobalamin deficiency progress. Thus, cobalamin deficiency should be excluded before initiating folate replacement therapy. 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 chronic hemolytic anemia should receive daily folate supplementation.
- HCY is elevated and MMA is normal in folate deficiency.
- Cobalamin deficiency should be ruled out before treatment with folate.

Other causes of megaloblastic anemia

In addition to folate and cobalamin deficiency, there are other rarer causes of megaloblastic anemias. Drugs that affect DNA synthesis cause megaloblastic anemia (5-fluorouracil–pyrimidine analog, azathioprine–purine analog, methotrexate–anti-folate, and hydroxyurea, zidovudine, and some anticonvulsant that also likely inhibit DNA synthesis). Nitrous oxide is associated with an acute megaloblastic anemia secondary to impaired cobalamin metabolism and abuse of this compound has been associated with psychosis and other neurologic defects.

Acquired pure red cell aplasia

Acquired pure red cell aplasia case

A 64-year-old female presents with fatigue and dyspnea on exertion, which has been progressive over the last 2 months. She is on no 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, 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.

Pure red cell aplasia (PRCA) is characterized by severe normochromic, normocytic or macrocytic anemia, reticulocytopenia, and the absence of hemoglobin-containing cells (some define as hemoglobinized cells including $<3\%$ of the nucleated marrow cells) in an otherwise-normal marrow aspirate or with maturation arrest at the proerythroblast stage (Figure 6-5).

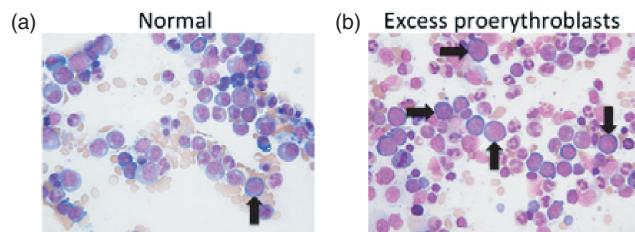


Figure 6-5 Pure red cell aplasia bone marrow aspirate with excess proerythroblasts. Arrowheads indicate proerythroblasts. Wright-Giemsa stained marrow aspirates of (a) normal patients and (b) pure red cell aplasia patients.

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 6-8). Alternatively, acquired PRCA can be classified by the pathophysiology of the anemia. There are three distinct mechanisms by which erythropoiesis can fail. 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. This can occur idiopathically or associated with an underlying disorder. 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 patients are usually between 6 and 36 months of age and present only with the insidious onset of pallor. The degree of anemia is variable. The differential diagnosis includes Diamond-Blackfan anemia and parvovirus B19 infection. Although the pathophysiology is not well characterized, most cases appear to be due to an immunoglobulin (IgG) antibody directed

against erythroblasts. The condition resolves spontaneously within several months with no sequelae.

Immunologic causes of acquired PRCA may be idiopathic in origin or secondary to an underlying disease. PRCA develops in ~5% of patients with thymoma, and conversely, thymoma occurs in ~10% of patients presenting with PRCA. The response to thymectomy in these cases is variable; a minority of patients achieves a complete remission after thymoma resection. PRCA also may be found in patients with underlying lymphoproliferative disorders (eg, large granular lymphocyte leukemia, chronic lymphocytic leukemia). Large granular lymphocyte (LGL) leukemia may be the most common underlying cause of acquired secondary PRCA. Approximately 20% of patients receiving ABO-mismatched bone marrow transplantation develop a prolonged RBC aplasia due to recipient isoantibodies, especially anti-A, against the donor RBCs; generally, the condition improves over time or with the development of graft-versus-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 are effective in some patients.

Parvovirus B19 is a common infection in children and causes 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, parvovirus persists at a high titer in the blood and marrow for 2-3 weeks and is then cleared. Because the normal life span of the RBC is ~120 days, infection does not 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 system is unable to clear the infection or in patients with shortened RBC survival (eg, sickle cell anemia or hereditary spherocytosis) in whom the anemic presentation is sometimes termed a “transient aplastic crisis.”

Many different drugs have been reported to cause PRCA, and discontinuing the drug sometimes resolves the PRCA. PRCA has been described as a result of the development of antierythropoietin antibodies during treatment with recombinant human ESAs. Although rare, this syndrome has occurred primarily after subcutaneous administration of Eprex (Janssen-Ortho, Toronto, Ontario, Canada), an erythropoietin- α product marketed outside of the United States. The number of ESA-associated PRCA cases peaked in 2001 and has since declined because of changes in the manufacturing, distribution, storage, and administration of Eprex. Because LGL leukemia may be present even in the absence of significant lymphocytosis, it is recommended that patients with idiopathic PRCA undergo lymphocyte immunophenotyping to look for LGL leukemia.

Table 6-8 Classification of pure red cell aplasia.

Congenital PRCA (Diamond-Blackfan anemia)
Acquired PRCA
Primary PRCA (likely immune-mediated mechanism)
Transient erythroblastopenia of childhood
Idiopathic
Secondary PRCA (immune consequence of an underlying disorder)
Thymoma
Hematologic malignancies (eg, chronic lymphocytic leukemia, large granular lymphocyte leukemia, multiple myeloma)
Solid tumors (eg, stomach, breast, lung, renal cell carcinomas)
Infections (eg, HIV, EBV, viral hepatitis)
Hematologic malignancies (eg, chronic lymphocytic leukemia, large granular lymphocytic leukemia)
Solid tumors (eg, stomach, breast, lung, renal cell carcinomas)
Infections (eg, HIV, EBV, viral hepatitis)
Collagen vascular diseases
Drugs and chemicals
Miscellaneous
Post-ABO incompatible bone marrow transplantation
Autoimmune chronic hepatitis or hypothyroidism
Parvovirus B19 (virus is directly cytotoxic to red blood cell precursors)
Myelodysplastic syndrome (hematopoietic stem cell that is unable to differentiate along the erythroid lineage)

EBV = Epstein-Barr virus; PRCA = pure red cell aplasia.

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. 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 (IFISH) 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, and parvovirus B19 DNA testing by polymerase chain reaction (serologies are not adequate).

PRCA caused by parvovirus B19 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.

Presumed immunologically mediated PRCA is treated with sequential trials of immunosuppressive therapies (eg, prednisone, cyclosporine, oral cyclophosphamide, mycophenolate mofetil, horse anti-thymocyte globulin (ATG), alemtuzumab, rituximab), which ultimately leads to remission in ~60%-70% of patients. No prospective randomized clinical trial data exist to support the use of one immunosuppressive agent over another, and we generally select an agent based on any identified underlying disorder, and in idiopathic cases, use prednisone or cyclosporine as first-line agents. A ~3-month trial of each immunosuppressive agent is reasonable to assess for response to therapy. Responding patients generally are treated for 3-6 months before immunosuppression is slowly tapered. Many patients will relapse after withdrawal of therapy and will require a long-term approach to immunosuppression, particularly if an underlying disorder (lymphoproliferative 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 (ie, iron overload and alloantibody formation). Supplemental ESAs are seldom effective, but they have been used in certain instances, such as after ABO-incompatible bone marrow transplantation.

Key points

- There are three pathophysiologic mechanisms of PRCA: immune-mediated, myelodysplasia, and parvovirus B19 infection in a susceptible host.
- 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.
- PRCA secondary to parvovirus B19 infection is treated with intravenous immunoglobulin.
- In the absence of clear myelodysplasia, parvovirus B19 PRCA is treated with courses of immunosuppressive agents.

Anemia associated with liver disease

Anemia and other hematopoietic abnormalities frequently are seen in patients with liver disease. The true incidence of anemia depends on the cause of liver disease, but it has been reported in up to 75% of patients with chronic liver disease. The etiology of anemia is multifactorial and likely reflects underproduction, blood loss, and increased destruction of RBCs. In alcoholic liver disease, concomitant folic acid deficiency may be seen and should be evaluated. Alcohol-induced pancreatitis may lead to decreased vitamin B12 absorption. Ethanol and its metabolites have been shown to inhibit erythroid production directly and may lead to acute or chronic anemia even in the absence of severe liver disease. In addition, erythropoietin production and erythropoiesis are suppressed by alcohol. Viral hepatitis may be associated with PRCA. GI blood loss is common in liver disease, especially in patients with esophageal varices. Shortened RBC survival also is noted in chronic liver disease. The decreased life span of RBCs in liver disease is at least partially explained by congestive splenomegaly, abnormal erythrocyte metabolism, and alterations in RBC membrane lipids. Changes in cholesterol composition lead to alterations in RBC surface area characterized by the target cells typically seen on review of the peripheral blood smear. Marked hemolytic anemia may develop in alcoholics with relatively mild liver disease. This condition, when accompanied by jaundice and hyperlipidemia, is termed Zieve syndrome. This syndrome may be self-limiting if the patient abstains from alcohol. Marked spur cells are noted on the blood smear in this condition, which has also been termed spur cell anemia (Figure 6-6). In the presence of underlying cirrhosis, spur cell anemia is likely irreversible without liver transplantation.

The typical anemia of liver disease is usually mild to moderate but may become more severe as cirrhosis, splenomegaly, and portal hypertension develop. The anemia is often macrocytic, but the MCV rarely exceeds 115 fl in the absence of megaloblastic changes in the bone marrow. The reticulocyte count is often minimally to moderately

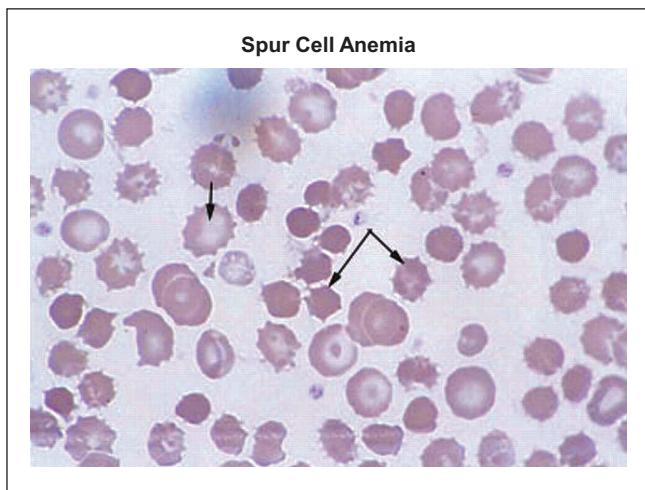


Figure 6-6 Note the acanthocytes (also known as spur cells and designated with arrows) and target cells.

increased in liver disease but may be suppressed by alcohol or concomitant iron deficiency. More marked reticulocytosis may be seen with hemorrhage or in spur cell anemia. Bone marrow cellularity often is increased and erythroid hyperplasia is observed. The peripheral blood smear often will show acanthocytes and target cells as the disease severity increases. Megaloblastosis may be seen in up to 20% of subjects. The treatment of anemia in liver disease is primarily supportive. If present, iron, vitamin B12, and folate deficiencies should be corrected. If ongoing hemolysis is noted, folate should be supplemented. Alcohol and other toxins should be avoided.

Sideroblastic anemias

The 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 that surrounds or rings the nucleus. This iron is in the form of mitochondrial ferritin. In both congenital and acquired sideroblastic anemia, the formation of ringed sideroblasts is due to insufficient production of protoporphyrin to utilize the iron that is delivered to erythroblasts or due to faults in mitochondrial function that affect iron pathways and impair iron incorporation into protoporphyrin. Acquired sideroblastic anemias may be clonal (MDS; Chapter 17) or secondary to alcohol, drugs (eg, isoniazid, chloramphenicol, linezolid), hypothermia, or copper deficiency. As a general rule, congenital sideroblastic anemias are microcytic and acquired sideroblastic anemias are macrocytic; however, this rule does not always hold.

Copper is an essential trace element that plays a role in numerous physiologic processes, including proliferation and

differentiation. Copper deficiency is rare in humans and generally is thought to result from inadequate intake (eg, patients on total parenteral nutrition without copper supplementation) or absorption (eg, postbariatric surgery, celiac disease, excessive zinc intake, congenital defect in copper transport called Menkes disease). Copper deficiency has been reported to result in anemia, neutropenia, and less commonly, thrombocytopenia. The anemia has been reported variably as microcytic, normocytic, or macrocytic. Importantly, copper deficiency can mimic an acquired MDS and can manifest with a macrocytic anemia and neutropenia and marrow morphology demonstrating ringed sideroblasts, dyserythropoiesis, dysmyelopoiesis, cytoplasmic vacuolization of both erythroid and myeloid precursors, and hemosiderin-laden plasma cells. In addition to the hematologic manifestations, copper deficiency can cause neurologic symptoms resembling subacute combined degeneration due to vitamin B12 deficiency.

The mechanism by which copper deficiency results in anemia or other cytopenias 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 by transferrin in the intestine and liver, respectively. Cytochrome C oxidase also requires copper as a cofactor and a decrease in this enzyme's activity potentially contributes to the development of ringed sideroblasts (reflecting mitochondrial iron accumulation) in some cases of copper deficiency. To diagnose copper deficiency, serum copper level is measured. A ceruloplasmin level also can be checked but may lack specificity.

Other underproduction anemias

The underproduction anemias discussed in this section generally are not categorized by MCV.

Anemia of cancer

The incidence of anemia in cancer patients is highly dependent on many variables, including cancer type, stage, and both present and past anticancer therapy. Its prevalence may exceed 90% in patients with advanced disease receiving chemotherapy. A large European survey of 15,000 patients showed that 67% of patients with cancer are anemic at diagnosis or become anemic (hemoglobin <12.0 g/dL) during the course of their treatment. The mechanisms underlying anemia of malignancy are complex, and numerous factors contribute to its development. Cytokine-mediated changes cause a relative decrease in erythropoietin production and a decrease in the response of erythroid precursors to

erythropoietin. As in AOCD, cytokines cause elevated hepcidin levels resulting in functional iron deficiency. Other causes of anemia in patients with cancer include effects of chemotherapy and radiotherapy, bone marrow infiltration, blood loss, autoimmune and microangiopathic hemolysis, and nutritional deficiencies.

A major change in the supportive care of cancer patients occurred with the availability of recombinant ESAs. Numerous studies have demonstrated a decrease in transfusion requirements among cancer patients receiving ESAs, with some studies also showing improvement in the quality of life in treated patients. Data, however, suggested that ESAs cause tumor growth and shortened survival in patients with advanced breast, head and neck, lymphoid, and non-small-cell lung cancer, especially when a hemoglobin of 12 g/dL was targeted. The safety of ESAs for the treatment of the anemia of cancer was again questioned in a recent large meta-analysis. This study analyzed individual patient data from 13,933 patients with cancer treated on 53 randomized controlled trials using ESAs versus standard of care. The analysis demonstrated a 17% increase in mortality in ESA-treated patients during the active study period. There was a 10% increase in mortality when the analysis was restricted to the studies that included patients treated with chemotherapy. A more recent analysis of the same data has shown that ESAs do not increase the risk of tumor progression if they are used according to guidelines, but there is a small increased risk of venous thromboembolic disease. The American Society of Hematology/American Society of Clinical Oncology guidelines on ESA use in cancer recommend using the lowest possible dose of an ESA that gradually will increase hemoglobin concentration to a level that avoids the need for transfusion and maintains hemoglobin levels <12 g/dL. ESAs are not recommended for patients receiving chemotherapy with curative intent. ESA should not be given in the treatment of anemia associated with malignancy in patients who are 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 improves the response rate with no increase in complications.

Key points

- Anemia is frequent in cancer patients and leads to a decreased quality of life.
- ESAs reduce transfusion requirements and improve quality of life in cancer patients.
- The use of ESAs in cancer patients requires careful patient counseling on the 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 nonhematopoietic or abnormal cells. The term myelophthusic is not used commonly in practice and more often is referred to descriptively as a marrow infiltrative process. Causes include tumors, granulomatous disorders, bone marrow fibrosis (due to a primary hematologic or numerous nonhematopoietic disorders), and lipid storage diseases. All of these causes may induce secondary marrow fibrosis. The peripheral blood smear in myelophthusic anemia often shows teardrop-shaped RBCs, immature leukocytes, nucleated RBCs, and occasional myeloblasts (ie, a leukoerythroblastic process). Rarely, carcinocytemia, defined as cancer cells in the circulating blood, is seen. Bone marrow biopsy may show frank tumor cells, Gaucher disease, or other infiltrating disorders and 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 magnetic resonance imaging 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. 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, and cranial nerve palsies. Osteopetrosis is caused by failure of osteoclast development or function, and mutations in at least 10 genes have been identified, accounting for 70% of cases. Severe cases are treated by bone marrow transplantation.

Anemia from malnutrition/anorexia nervosa

Data from survivors of World War II concentration camps have provided evidence that prolonged starvation can lead to a normochromic, normocytic anemia. The bone marrows of 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 6-7).

Anemia associated with endocrine disorders

In general, the anemia accompanying most endocrine disorders is mild, often asymptomatic, and likely overshadowed by the clinical effects of the specific hormone deficiency. In fact, in some cases, the anemia actually may be considered physiologic due to the decreased oxygen requirements accompanying some of these hormone deficiencies.

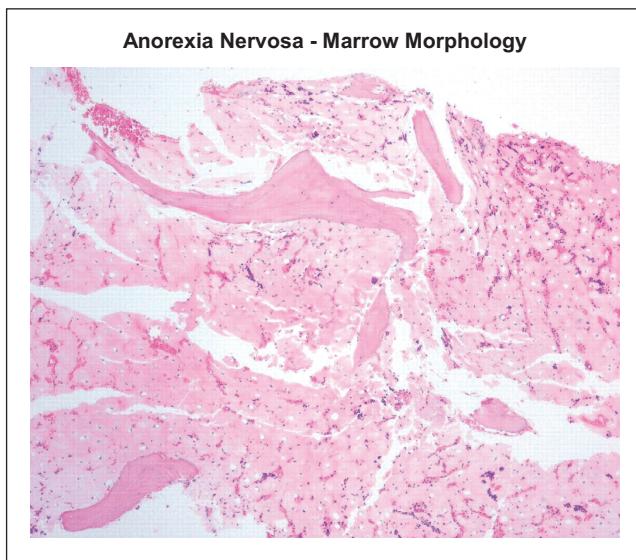


Figure 6-7 A marrow biopsy is shown illustrating almost complete replacement of the marrow by the hyaluronic acid extracellular matrix material. Hematopoietic elements and fat cells are markedly decreased. (H&E 4x)

Pituitary deficiency may be associated with a mild normochromic normocytic anemia. The bone marrow of such patients is usually hypoplastic and resembles that seen in other marrow failure states. Anemia most likely is due to secondary deficiency of hormones produced by endocrine organs that normally are stimulated by the anterior lobe of the pituitary (thyroid hormone, androgens, or cortisol) and modulate erythropoietin production. The anemia responds to appropriate hormone replacement.

Patients with primary hypothyroidism may be anemic. The anemia is felt to be secondary to an absence of erythropoietin-stimulated erythroid colony formation from lack of triiodothyronine (T₃), thyroxine (T₄), and reverse triiodothyronine (rT₃). The anemia is usually normochromic and normocytic, and hemoglobin values usually are not <8 g/dL. Macrocytosis may occur in patients with autoimmune hypothyroidism, particularly if there is coexistent B12 deficiency, folate deficiency, or hemolysis. Conversely, microcytosis can occur in women with concomitant iron deficiency from menorrhagia, which may be seen in myxedema. There is a well-recognized association between hypothyroidism and PA, so patients with either disorder should be screened for the other. The response to thyroid replacement is typically sluggish, and it may take months before the anemia resolves. Unlike patients with hypothyroidism, patients with hyperthyroidism are only rarely anemic, and anemia in this condition is typically microcytic and poorly understood.

Hypogonadism usually results in a decrease of 1-2 g/dL in hemoglobin concentration. Androgens stimulate increased erythropoietin production and can increase the effects of erythropoietin on the developing erythron. Thus, men typically

have higher hemoglobin concentrations than age-matched women. The hemoglobin values of men treated with antiandrogen therapy for prostate cancer typically falls by 1-2 g/dL.

Patients with Addison's disease may have a normochromic normocytic anemia that is responsive to ESAs or glucocorticoids. The mild decrease in RBC mass may be masked by a concomitant decrease in plasma volume, leading to a normal hemoglobin concentration. When replacement with glucocorticoid therapy initially is begun, the anemia may become apparent as the plasma volume is restored. Androgens sometimes are used to correct anemias due to marrow hypoproduction in such conditions as myelodysplasia and myelofibrosis.

Anemia is more severe and occurs at an earlier stage in patients with diabetic nephropathy compared with patients with renal failure from other causes. The exact mechanism for this finding remains uncertain.

Anemia associated with pregnancy

Anemia is a common complication of pregnancy. Normally, the RBC mass increases ~20%-30% during gestation, while the plasma volume increases ~40%-50%, resulting in a normochromic and normocytic anemia. This so-called physiologic anemia of pregnancy reaches a nadir at ~30 weeks. Because the RBC mass continues to increase after 30 weeks, when plasma volume expansion has reached a plateau, the hematocrit may rise somewhat after 30 weeks.

Definitions of pathologic anemia during pregnancy vary. United Kingdom guidelines define anemia in pregnancy as a hemoglobin <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. The evaluation and workup of anemia in pregnant patients should mirror nonpregnant patients. Additionally, special consideration should be paid to any proposed therapies since any treatment has effects on both the mother and developing fetus.

Iron deficiency during pregnancy is common, especially in non-Western cultures. A full-term pregnancy requires a total of ~1 g of iron, which includes ~300 mg of iron for the fetus, ~200 mg of maternal iron lost through various normal routes of excretion, and ~500 mg of iron for the expanding maternal RBC mass. Additionally, iron is secreted in breast milk for use by the developing infant. Folate requirements also increase during pregnancy, and megaloblastic anemia has been reported, especially 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. Of note, serum 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 developing worsening pancytopenia or relapse during pregnancy.

Key points

- Anemia in pregnancy is due in part to an imbalance between the expansion of the plasma volume and the RBC mass.
- Iron deficiency and folate deficiency are important causes of anemia in pregnancy.
- The evaluation of anemia in pregnancy should mirror the evaluation of anemia in nonpregnant patients.

Anemia of the elderly

Recently, findings from the National Health and Nutrition Examination Study (NHANES III) indicated that 11.0% of men and 10.2% of women over the age of 65 were anemic (based on the definition of anemia as hemoglobin <13 g/dL for men and <12 g/dL for women). Other studies have supported this finding, as well as the higher prevalence rates of anemia in elderly African Americans. Anemia is an independent risk factor for cognitive decline and is associated with decreased bone density, decreased muscle strength, and decreased physical performance in elderly patients. The presence of anemia, either with or without other chronic illnesses, is associated with increased hospitalization, morbidity, and mortality.

Analysis of data from NHANES III showed that approximately two-thirds of the cases of anemia were attributable to iron deficiency, other nutritional deficiencies, chronic inflammatory illnesses, and renal insufficiency. On the basis of information available in the database, however, 34% of cases were unexplained. Several studies have attempted to elucidate the mechanisms for this group of unexplained anemias. Investigators have suggested that a variety of age-related physiologic changes may contribute to this process, including blunting of the hypoxia-driven ESA response, decrease in sex steroids, and alterations in bone marrow stem cells and cellularity. Because it is likely that anemia is multifactorial in a given individual, 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 6-9.

Key points

- Anemia is common in elderly patients and often multifactorial.
- In two-thirds of patients, the anemia is caused by hematologic deficiency or AOCD and is unexplained in about one-third of patients.
- Functional impairment and increased morbidity and mortality have been demonstrated in elderly anemic patients.

Table 6-9 Evaluation of anemia in the elderly for the clinical hematologist: a practical approach.

Always useful

1. Anemia-oriented history and physical examination, with particular emphasis on comorbid conditions associate with anemia and drug history
2. CBC/differential/platelet, absolute reticulocyte count, smear review
3. Tests of iron stores (Fe, TIBC, ferritin)
4. Serum cobalamin and folate levels
5. Chemistry panel (including calculated creatinine clearance)
6. TSH

Sometimes useful

1. Serum testosterone
2. Serum erythropoietin (with caveat for what represents a “normal” erythropoietin in an elderly person)
3. Tests of inflammation (ESR, C-reactive protein)
4. Methylmalonic acid, serum homocysteine
5. RBC folate level
6. Bone marrow aspiration and biopsy, cytogenetics

Modified from Guralnik JM, Ershler WB, Schrier SL, et al. Anemia in the elderly: a public health crisis in hematology. *Hematology Am Soc Hematol Educ Program*. 2005:531.

CBC = complete blood count; ESR = erythrocyte sedimentation rate; Fe = iron; RBC = red blood cell; TIBC = total iron-binding capacity; TSH = thyroid-stimulating hormone.

Anemia associated with HIV infection

Anemia is the most prevalent hematologic abnormality associated with HIV infection. Not surprisingly, anemia prevalence increases with HIV disease progression. Several studies have shown that anemia is associated independently with decreased survival and decreased quality of life in HIV-infected patients. Anemia in HIV-infected patients is usually multifactorial, and the most likely etiologies depend on the stage of the HIV infection and the medications the patient is receiving. Zidovudine and trimethoprim-sulfamethoxazole are commonly associated with bone marrow suppression and macrocytosis. Infections 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 poor nutrition. Patients with HIV are at risk for hemolysis due to a variety of mechanisms, 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. The HIV virus itself 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 the inclusion of zidovudine in the regimen does not appear to diminish the beneficial effect of HAART on anemia. In addition to HAART, the management of anemia in HIV patients should include correction of nutritional deficiencies and treatment of infections. ESAs have been shown to reduce transfusion requirements in HIV patients with baseline erythropoietin 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 medications.
- Macrocytosis is more common than anemia in patients treated with zidovudine.
- HAART reduces the incidence and degree of anemia in HIV-infected patients.

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Hemolytic anemias

Charles T. Quinn and Charles H. Packman

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CHAPTER
07

Hemolytic anemias

Charles T. Quinn and Charles H. Packman

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Introduction

Hemolysis is the accelerated destruction, and hence decreased life span, of red blood cells (RBCs). The bone marrow's response to hemolysis is increased erythropoiesis, evidenced by reticulocytosis. If the rate of hemolysis is modest and the marrow is able to completely compensate for the decreased RBC life span, then 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.

Clinically, hemolytic anemia produces variable degrees of fatigue, pallor, and jaundice. Splenomegaly occurs in some conditions. The complete blood count shows anemia and reticulocytosis that depend on the severity of hemolysis and the degree of bone marrow compensation. Secondary chemical changes include indirect hyperbilirubinemia, increased urobilinogen excretion, and elevated lactate dehydrogenase (LDH). Decreased serum haptoglobin levels and increased plasma free hemoglobin also may be detected. Because free hemoglobin scavenges nitric oxide, esophageal spasm or vascular sequelae such as non-healing skin ulcers can occur. Chronic intravascular hemolysis produces hemosiderinuria, and chronic extravascular hemolysis increases the risk of pigmented (bilirubinate) gallstones.

The hemolytic anemias can be classified in different yet complementary ways (Table 7-1). They can be inherited (eg, sickle cell disease or hereditary spherocytosis) or acquired

Table 7-1 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

RBC = red blood cell.

(eg, autoimmune or microangiopathic). Alternatively, they can be characterized by the anatomic site of RBC destruction: extravascular or intravascular. Extravascular hemolysis, erythrocyte destruction 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), and some are predominantly extravascular (eg, hereditary spherocytosis). Others have substantial components of both, such as sickle cell anemia in a young child with an intact spleen.

The hemolytic anemias can be classified according to whether the cause of hemolysis is intrinsic or extrinsic to the RBC—whether the damage occurs from within or without. Intrinsic causes of hemolysis include abnormalities in

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Off-label drug use: Dr. Quinn: not applicable. Dr. Packman: Rituximab, cyclophosphamide, azathioprine, mycophenolate mofetil, cyclosporine and danazol in the treatment of autoimmune hemolytic anemia.

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, paroxysmal nocturnal hemoglobinuria (PNH) is an acquired intrinsic RBC disorder, and congenital thrombotic thrombocytopenia purpura (TTP) is an inherited cause of extrinsic hemolysis. In this chapter, hemolytic anemias will be divided into intrinsic and extrinsic forms.

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 hemoglobin

Hemoglobin (Hb) is the oxygen-carrying protein within RBCs. It is composed of four globular protein subunits, called globins, and four oxygen-binding heme groups, which are attached to each globin. The two main types of globins are the α globins and the β -globins, which are made in essentially equivalent amount in precursors of RBCs. Normal adult Hb (Hb A) has two α -globins and two 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 analogs of the α -globins and β -globins encoded by separate genes.

Hb production

The gene cluster for the non- α (β -like) globins is on chromosome 11 and includes an embryonic ϵ -globin gene, the two fetal globin genes γ (A^{γ} and G^{γ}), and the two adult δ - and β -globin genes. The cluster of α -like globin genes is on chromosome 16 and includes the embryonic ζ -globin gene and the duplicated α -globin genes (α_2 and α_1), which are expressed in both fetal and adult life. Both clusters also contain nonfunctional genes (pseudogenes) 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 bind erythroid-specific and nonspecific DNA binding proteins and serve as a “master switch,”

inducing expression within the downstream gene cluster. In addition to binding specific transcriptional regulatory proteins, the 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 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 Hb 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 Hb 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 Hb (Hb F) at the expense of Hb A could successfully treat sickle cell disease and β -thalassemia.

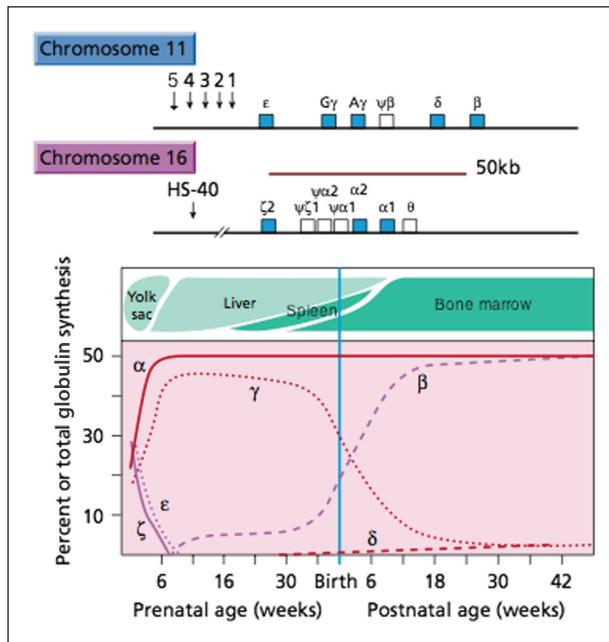


Figure 7-1 Hemoglobin gene clusters and developmental hematopoiesis. The organization of the α - and β -globin gene clusters are shown at the top of the figure. The bottom portion of the figure illustrates the developmental changes in Hb production, both by the site of production of blood and changes in the proportions of the different globins. Modified with permission from Stamatoyannopoulos G, Majerus PW, Perlmutter RM, et al., eds. *The Molecular Basis of Blood Diseases*. 3rd ed. Philadelphia, PA: WB Saunders; 2001.

Hb structure

Hb is a tetramer consisting of two pairs of globin chains. Heme, a complex of ferrous iron and protoporphyrin, is linked covalently to each globin monomer and can bind reversibly one oxygen molecule. The molecular weight of Hb is approximately 64 kd. 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 Hbs are Hb A ($\alpha_2\beta_2$), Hb A₂ ($\alpha_2\delta_2$), and Hb F ($\alpha_2\gamma_2$).

When Hb is deoxygenated, there are substantial changes in the structures of the individual globins and the Hb 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 Hb leads to disruption of the salt bonds and transition to a relaxed (R) conformation.

Hb function

Hb enables RBCs to deliver oxygen to tissues by its reversible binding of oxygen. With the sequential binding of one oxygen molecule to each of the four heme groups, the salt bonds are progressively broken, which increases the oxygen affinity of the remaining heme moieties. Cooperativity 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 Hb 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 two 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 increases 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 Hb, thereby

reducing its oxygen affinity. The intraerythrocytic molar concentrations of 2,3-BPG and Hb 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 Hb; however, this does not play a significant role in carbon dioxide transport. It recently has been reported that Hb binds nitric oxide in a reversible manner. The participation of Hb in modifying regional vascular resistance through this mechanism has been proposed.

Disorders of Hb

Disorders of Hb can be classified as qualitative or quantitative disorders. Qualitative abnormalities of Hb arise from mutations that change the amino acid sequence of the globin, thereby producing structural and functional changes in the Hb. There are four ways in which Hb can be qualitatively abnormal: (i) decreased solubility, (ii) instability, (iii) altered oxygen affinity, and (iv) altered maintenance of the oxidation state of the heme-coordinated iron. Hemolytic anemia results from decreased solubility and instability of Hb. Qualitative Hb disorders often are referred to as hemoglobinopathies, even though the term technically can apply to both qualitative and quantitative disorders. Quantitative Hb 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 will be a relative excess of α -globins. Such imbalanced production of α - and β -globins damages RBCs and their precursors in the bone marrow. These quantitative Hb disorders are called thalassemias. Both qualitative and quantitative disorders of Hb can be subdivided by the particular globin that is affected; for example, there are α -thalassemias and β -hemoglobinopathies, among others. We will begin this chapter with a review of the thalassemias and end with a discussion of several of the common qualitative Hb disorders.

Thalassemia

Clinical case

PC is a healthy 48-year-old female of African descent who 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 10-11 g/dL with a mean corpuscular volume (MCV) ranging from 69-74 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

Clinical case (continued)

with an MCV of 71 fL and mean corpuscular hemoglobin (MCH) of 23 pg. Additional laboratory studies include a transferrin saturation of 32% and ferritin 490 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. If a mutation decreases the synthesis of one globin, α or β , it produces a relative excess of the other and an imbalance between the two. For example, if β -globin synthesis is diminished by a mutation, there will be a relative excess of α -globins. Such imbalanced production of α - and β -globins results in damage to precursors of RBCs in the bone marrow. This damage occurs largely because the excess unpaired globin is unstable, and it 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 is the result. 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 pathophysiological process is called ineffective erythropoiesis.

The thalassemias can be described simply by two 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 two systems can be used together, giving α -thalassemia major or β -thalassemia intermedia, for example.

β -Thalassemias

β -Thalassemia is prevalent in the populations of the Mediterranean region, the Middle East, India, Pakistan, and

Southeast Asia. It is somewhat less common in Africa. It is rarely encountered in Northern European Caucasians.

Molecular basis

β -Thalassemia results from >150 different mutations of the β -globin gene complex (Figure 7-2). Abnormalities have been identified in the promoter region, messenger RNA (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). The clinical phenotype of patients with β -thalassemia therefore is determined largely by the number and severity of the abnormal alleles they inherit. β -thalassemia major (Cooley anemia) is almost always caused by homozygous β^0 thalassemia. Patients who are compound heterozygotes for thalassemic alleles, at least one of which is β^+ , usually will have thalassemia intermedia. Patients with thalassemia minor usually are heterozygous, carrying a single β -thalassemia allele (thalassemia trait), but some patients who are compound heterozygotes for

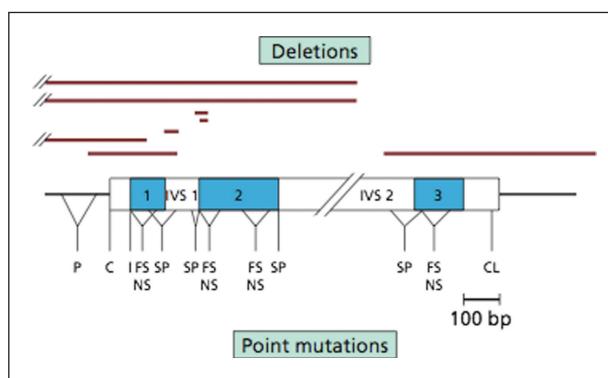


Figure 7-2 Common β -thalassemia mutations. The major classes and locations of mutations that cause β thalassemia are shown. From Stamatoyannopoulos G, Majerus PW, Perlmutter RM, et al., eds. *The Molecular Basis of Blood Diseases*. 3rd ed. Philadelphia, PA: WB Saunders; 2001. C = CAP site; CL = RNA cleavage [poly(A)] site; FS = frameshift; I = initiation codon; NS = nonsense; P = promotor boxes; SP = splice junction, consensus sequence, or cryptic splice site.

very mild β^+ alleles also may have α -thalassemia minor phenotype.

Pathophysiology

In β -thalassemia, decreased β -chain synthesis leads to impaired production of the $\alpha_2\beta_2$ tetramer of Hb A. The reduction in Hb A in each of the circulating RBCs results in hypochromic, microcytic RBCs with target cells, a characteristic finding in all forms of β -thalassemia. In addition, aggregates of excess α -chains precipitate and form inclusion bodies, leading to premature destruction of erythroid precursors in the bone marrow (ineffective erythropoiesis). In more severe forms, circulating RBCs also may contain inclusions, leading to early clearance by the spleen. Splenomegaly, due to extramedullary hematopoiesis, may further contribute to the shortened RBC survival. The precipitated α -globin chains and products of degradation also may induce changes in RBC metabolism and membrane structure, leading to shortened RBC survival. In severe β -thalassemia, an increased level of Hb F is distributed to varying degrees within the erythrocytes, yielding a heterogeneous pattern that persists past early infancy. It has been suggested that these cells have less α chain precipitation due to formation of $\alpha_2\gamma_2$ tetramers (Hb F). Therefore, this cell population survives longer in the bone marrow and circulation. 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.

Clinical features

The clinical manifestations of β -thalassemia are quite heterogeneous and depend on the extent of β -globin chain production as well as the coinheritance of any other abnormalities affecting α - or γ -globin synthesis or structural abnormalities of Hb (eg, Hb S).

β -Thalassemia major, the clinical syndrome denoted Cooley anemia, results from homozygous or compound heterozygote genotypes that lead to absent or severe deficiency in β -chain synthesis. Symptoms are usually evident within the first 6 months of life as the Hb F level begins to decline. In the absence of adequate RBC transfusions, the infant will experience failure to thrive and a variety of clinical findings. Erythroid expansion leads to widening of the bone marrow space, thinning of the cortex, and osteopenia, predisposing 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 may ameliorate severe anemia and suppress ineffective erythropoiesis. Chronic

transfusion can reduce growth retardation and skeletal abnormalities, but with less intensive regimens, splenomegaly is still observed. Iron overload may occur secondary to increased intestinal absorption as well as RBC transfusions. Iron chelation therapy has improved patient outcome by preventing or delaying the substantial morbidity and mortality of iron overload. Ineffective chelation results in progressive iron overload and consequent heart, liver, and endocrine organ failure. An increased frequency of *Yersinia enterocolitica* bacteremia is associated with iron overload and chelation therapy.

Patients with β -thalassemia intermedia are moderately anemic but do not require regular, chronic transfusions by definition. Their disease usually results from the inheritance of 2 thalassemic genes, at least one of which is a β^0 allele. These patients exhibit a wide spectrum of clinical findings. Many have splenomegaly and prominent bony expansion. Some patients require intermittent transfusion support, whereas others are asymptomatic despite significant anemia. Patients with significant ineffective erythropoiesis have increased intestinal absorption of iron. Consequently, even in the absence of transfusion, some patients may have iron overload. An increased incidence of cerebral thrombosis and venous thromboembolism has been reported in β -thalassemia major and β -thalassemia intermedia following splenectomy.

β -Thalassemia 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 Hb F to Hb A production progresses.

Heterozygotes with the rare $\epsilon\gamma\delta\beta$ deletion present with moderately severe microcytic anemia in the neonatal period that improves during the first several months of life. In the adult, the hematologic findings are those of β -thalassemia trait, except that the Hb electrophoresis shows normal Hb A₂ levels (see the section "Laboratory findings").

Laboratory findings

A child with β -thalassemia major who is not receiving transfusions will have severe anemia. Peripheral blood smear findings include anisopoikilocytosis, target cells, severe hypochromia, 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 Hb F ($\alpha_2\gamma_2$) and variable elevation of Hb A₂ ($\alpha_2\delta_2$). Hb A is absent in homozygous β^0 thalassemia. A variable degree of anemia with hypochromic, microcytic cells and targets is observed in β -thalassemia intermedia. Patients with β -thalassemia trait usually have Hb values between 9 and 11 g/dL, and microcytic, hypochromic RBCs and target cells

usually are seen. Basophilic stippling is variable. The MCV is usually <70 fL, the MCH is reduced, and the reticulocyte count can be mildly elevated. Hb A₂ levels are elevated >3.5% (usually 4%-7%), and Hb F levels may be mildly increased. In δβ thalassemia trait, the Hb A₂ level is normal, but the Hb F level is elevated (typically 5%-10%).

Management of the β-thalassemias

RBC transfusion has been the mainstay in the management of β-thalassemia major. With initiation of a chronic transfusion regimen early in childhood, devastating clinical manifestations can be avoided. A transfusion program maintaining pre-transfusion Hb levels at 9.5 g/dL can be as effective as more aggressive regimens (ie, those maintaining Hb levels >11 g/dL). Iron chelation therapy with deferasirox or deferoxamine is usually initiated when serum ferritin levels reach approximately 1,000-1,500 ng/mL following 12-18 months of scheduled transfusions. Vitamin C supplementation can enhance iron excretion. Splenectomy is performed to alleviate abdominal symptoms or increased transfusion requirements but usually is delayed until after the age of 5 years because of the risk of sepsis secondary to encapsulated organisms. Allogeneic bone marrow transplantation from a histocompatibility (human leukocyte antigen [HLA]-compatible) sibling has been performed in >1,000 patients and is now curative in most. The outcome has been influenced by the age of the patient, the presence of liver disease, and the extent of iron overload. Graft-versus-host disease represents the most common long-term complication. Recent studies exploring non-myeloablative or unrelated donor transplantation are encouraging, even in patients with prior iron loading (for whom chelation therapy before transplantation is advised) or concomitant hepatitis C virus (HCV) infection. Many adults with thalassemia major have chronic HCV infection resulting from contaminated RBC products that they received before the early 1990s. Treatment with γ-interferon and ribavirin may be complicated by hemolysis resulting from ribavirin. Investigational approaches include using erythropoietin, hydroxyurea, decitabine, and butyrate compounds to increase Hb F levels. Children with β-thalassemia intermedia who have moderately severe anemia or bony expansion also may benefit from regular RBC transfusions. Individuals with the β-thalassemia trait do not require therapy but should be identified to reduce the risk of inappropriate iron supplementation and inform reproductive choices.

α-Thalassemias

There is a high prevalence of α thalassemia in Africa, the Mediterranean region, Southeast Asia, and, to a lesser extent, the Middle East.

Molecular basis

Duplicated copies of the α-genes normally are present on each chromosome 16, making the defects in α thalassemia more heterogeneous than in β thalassemia. The α⁰ thalassemias result from loss of linked α-genes on the same chromosome, denoted as --/αα. Deletions of the α-genes or a deletion in HS-40, the upstream regulatory region, account for most of the α⁰ thalassemia mutations. 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 one in three African Americans. The --/αα genotype of α thalassemia trait (deletions in the *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. Hb Constant Spring is a nondeletion α⁺ thalassemia, common in Southeast Asia, resulting from a mutation that affects termination of translation and results in abnormally elongated α-chains.

Pathophysiology

As in the β-thalassemias, the imbalance of globin chain synthesis results in decreased Hb synthesis and microcytic anemia. Excess γ- and β-chains form tetramers termed Hb Bart and Hb H, respectively. These tetramers are more soluble than unpaired α globins and form RBC inclusions only 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 (γ₄), and there may be persistence of embryonic Hbs. 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 tissue hypoxia, resulting in edema, congestive heart failure, and death. Hb H disease results from the coinheritance of α⁰ thalassemia and α⁺ thalassemia (--/-α or α⁰ thalassemia and a nondeletional form of α-thalassemia (--/α^Tα), such as Hb Constant Spring (--/α^{CS}α). The excess β-chains form Hb H (β₄) that is unstable, causing precipitation within circulating cells and hemolysis. Patients have moderately severe hemolytic anemia.

Hb H 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 (ATMDS). 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 features

In contrast to β -thalassemias, α -thalassemias 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 7-3). Homozygous α^0 thalassemia ($-/-$) results in the Hb Bart hydrops fetalis syndrome. The lack of

Hb F 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. Moreover, the mother often experiences morbidity from polyhydramnios. The clinical manifestations are variable in Hb H disease ($-/-\alpha$), with severe forms demonstrating transfusion dependence and other individuals having a milder course. As in β -thalassemia, splenomegaly occurs commonly in the anemic patient. The homozygous state for Hb Constant Spring results in moderate anemia with splenomegaly. Hb H–Constant Spring ($- - \alpha^{CS}\alpha$) is typically more severe than classical Hb H disease ($-/-\alpha$). Thalassemia trait (two-gene deletion α thalassemia) occurs in two forms: α^0 thalassemia trait ($-/\alpha\alpha$) or homozygosity for α^+ thalassemia (α/α). Individuals with thalassemia trait have a lifelong mild microcytic anemia. Heterozygotes for α^+ thalassemia ($\alpha/\alpha\alpha$), so-called silent carriers, are clinically normal.

Laboratory features

The blood smear in Hb Bart hydrops fetalis syndrome reveals markedly abnormal RBC morphology with anisopoikilocytosis, hypochromia, targets, basophilic stippling, and nucleated RBCs. The Hb electrophoresis reveals approximately 80% Hb Bart and the remainder Hb Portland ($\zeta_2\gamma_2$). Hb H disease is characterized by anisopoikilocytosis and hypochromia with elevated reticulocyte counts. Hb electrophoresis reveals 5%–40% of the rapidly migrating Hb H. Supravital staining with brilliant cresyl blue will reveal inclusions representing in vitro precipitation of Hb H. In newborns with α^0 thalassemia trait, the Hb electrophoresis reveals 2%–5% Hb Bart and microcytosis (<95 fL). Children and adults heterozygous for α^0 thalassemia ($-/\alpha\alpha$) or homozygotes for α^+ thalassemia (α/α) have mild anemia with hypochromic, microcytic RBCs and target cells. The RBC indices are similar to those of β -thalassemia trait, but the Hb electrophoresis is normal (or shows a reduction in Hb A₂). The high prevalence of the $-\alpha/-\alpha$ genotype in African Americans is noteworthy. About 2%–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. Minimal or no anemia or morphologic abnormalities of RBCs are observed in silent carriers, that is, heterozygous α^+ thalassemia ($\alpha/\alpha\alpha$), and the Hb electrophoresis is normal.

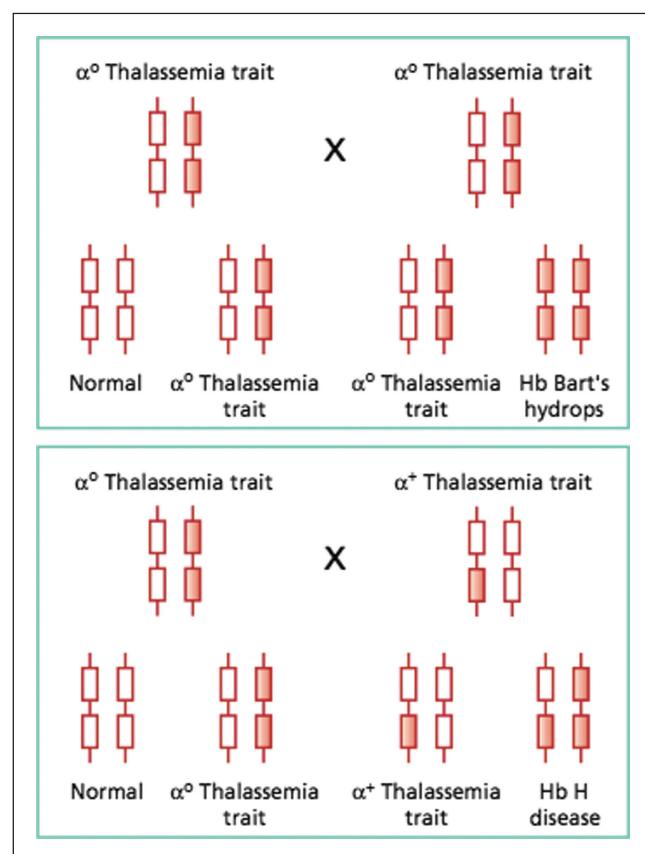


Figure 7-3 Genetics of α -thalassemia. The α -globin genes are represented as boxes. The red α -globin genes represent deletions or otherwise inactivated α -genes. The open boxes represent normal α -genes. The terms α^0 and α^+ thalassemia are defined in the text. The potential offspring of two parents with α^0 thalassemia trait is shown in the upper panel. The potential offspring of one parent with α^0 thalassemia trait and the other with α^+ thalassemia trait is shown in the lower panel (note the lack of hemoglobin Bart hydrops fetalis in these offspring). From Stamatoyannopoulos G, Majerus PW, Perlmuter RM, et al., eds. *The Molecular Basis of Blood Diseases*. 3rd ed. Philadelphia, PA: WB Saunders; 2001.

Management of the α -thalassemias

A fetus with homozygous α^0 thalassemia can be rescued with intrauterine transfusion, followed by postnatal chronic transfusions or stem cell transplantation. Patients with Hb H

disease usually require no specific interventions. For those patients with splenomegaly and significant anemia, splenectomy may prove useful. Some patients, especially those with Hb H–Constant Spring, require intermittent or chronic RBC transfusions. 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 Bart hydrops fetalis and Hb H disease.

Clinical case (continued)

The patient presented in this case likely has the homozygous state for α^+ thalassemia ($-/\alpha^-/\alpha^-$). 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. 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 β -thalassemias are caused by >150 different mutations, usually point mutations, with a wide variety of genetic abnormalities documented.
- Patients with thalassemia major require transfusion support, experience iron overload, and may benefit from splenectomy. A spectrum of clinical manifestations is observed in thalassemia intermedia, whereas the carrier state has no associated symptoms.
- The hemoglobin electrophoresis in β -thalassemia reveals increased levels of hemoglobin A₂ and variably increased hemoglobin F.
- The α -thalassemias are primarily due to DNA deletions. Four α -genes normally are present, so multiple phenotypes are possible when gene deletions occur.
- Homozygous α^0 thalassemia manifests in fetal life with the formation of hemoglobin Bart (γ_4) and hydrops fetalis.
- The clinical manifestations in hemoglobin H disease are variable, with some affected individuals requiring transfusions and others less symptomatic.
- α -Thalassemia trait is characterized by mild anemia with microcytic indices and a normal hemoglobin electrophoresis.

Sickle cell disease

Clinical case

A 17-year-old African American male with homozygous sickle cell anemia (Hb SS) 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, ill appearing, 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/ μ L. 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 (CPK) 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 Hb (Hb S) was the first Hb variant discovered. It has been well characterized on a 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, and approximately 5% of individuals in parts of the Mediterranean region, the Middle East, and North Africa. In Hb S, a hydrophobic valine is substituted for the normal, more hydrophilic glutamic acid at the sixth residue of the β -globin chain (Figure 7-4). This substitution is due to a single nucleotide mutation (GAG/GTG) in the sixth codon of the β -globin gene. Heterozygous inheritance of Hb S offers a degree of protection from severe malaria infection. This has been offered as an explanation for the evolutionary selection of the Hb S 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); homozygous inheritance or compound heterozygous inheritance with another mutant β -globin gene results in disease. The *sickle cell syndromes* include all conditions in which 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

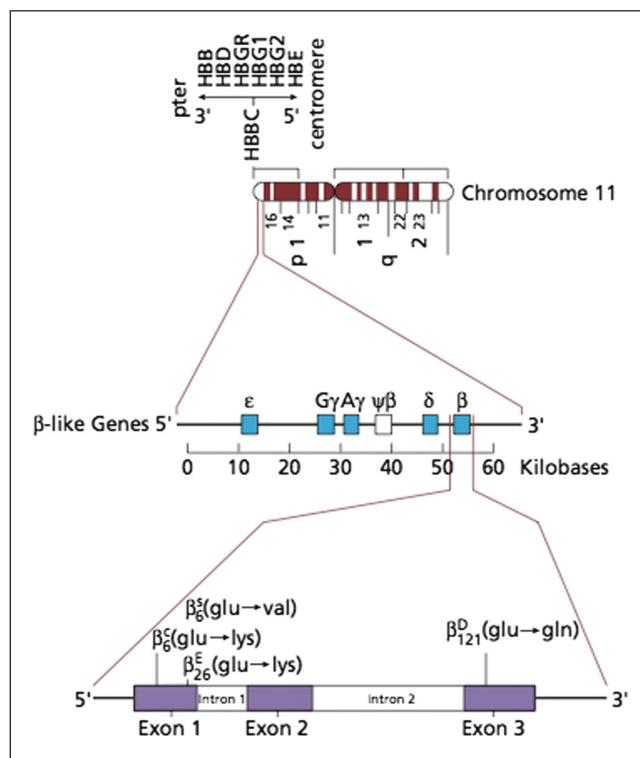


Figure 7-4 Common β -globin variants. The locations of the mutations within the chromosome (top), the β -globin cluster (middle), and the β -globin gene itself (bottom) are shown for four common β -globin variants.

hemolytic anemia and vaso-occlusive pain (not sickle trait): homozygous sickle cell anemia (Hb SS), sickle-Hb C disease (Hb SC), sickle- β^0 thalassemia (Hb S β^0), and sickle- β^+ thalassemia (Hb S β^+). Less common Hb 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 (Hb AS) occurs in 8%-9% of the African American population and is best regarded as a benign condition, despite 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. This simple heterozygous state generally has an Hb 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 coinherited 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 Hb S occurs, the normal conformational change of the tetramer exposes on its external surface a hydrophobic β_6 valine (instead of the hydrophilic glutamate of Hb A), 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 Hb S, the type and fractional content of other Hbs present (particularly Hb F), 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 Hb abnormalities on the effects of Hb S. For example, the coexistence of α thalassemia reduces the hemolytic severity as well as the risk of cerebrovascular accidents. High levels of fetal Hb (Hb F) may substantially reduce symptoms as well as clinical consequences. Compound heterozygosity for a second abnormal Hb (eg, Hb C, D, or E) or β -thalassemia also modifies some of the manifestations of disease (discussed later in this section; Table 7-2).

Table 7-2 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

*Hb A_2 can be increased in the presence of Hb S, even in the absence of β -thalassemia. The classical findings are shown here.

†There will be 50% hemoglobin C that migrates near hemoglobin A_2 on alkaline gel electrophoresis or isoelectric focusing.

HPFH = hereditary persistence of fetal hemoglobin.

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 also have 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 (Hb F) production. The β^S gene has been found to be associated with five 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 five 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. 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 (VCAM-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 have 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 contributes to the hypercoagulability by providing procoagulant surfaces. The correlation of elevated white blood cell counts to increased mortality and adverse outcomes identified by epidemiologic studies of sickle cell disease patients suggests 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 (G-CSF). These findings 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 Hb S in erythrocyte hemolysates. Historically, cellulose acetate electrophoresis at alkaline pH was used to separate Hb A, Hb A₂, and Hb S, and citrate agar electrophoresis at acidic pH was used to separate comigrating Hb D and Hb C from Hb S and Hb A₂, respectively. Currently, high-performance liquid chromatography (HPLC) and isoelectric focusing are used in most diagnostic laboratories to separate Hbs. In both Hb SS and S β^0 thalassemia, no Hb A is present. In Hb SS, however, the MCV is normal, whereas in Hb S β^0 thalassemia, the MCV is reduced. Hb A₂ is elevated in S β^0 thalassemia, but it also can be nonspecifically elevated in the presence of Hb S, so an elevation of A₂ alone cannot reliably distinguish Hb SS from S β^0 thalassemia. In sickle cell trait and S β^+ thalassemia, both Hb S and Hb A 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 will reveal the presence of irreversibly sickled cells in Hb SS and Hb S β^0 thalassemia (Figure 7-5), but only rarely in S β^+ thalassemia and Hb SC. Turbidity tests (for Hb S) are positive in all sickle cell syndromes, including Hb AS (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 Hb S, so they do not differentiate sickle cell disease from sickle cell trait. Therefore, they have limited utility. Sickled 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 clinical manifestations of sickle cell disease. The erythrocyte life span is shortened from the normal 120 days to approximately 10-25 days, resulting in moderate to severe hemolytic anemia, with a steady-state mean Hb concentration of 8 g/dL (ranging from 6 to 9 g/dL) in Hb SS disease. The anemia is



Figure 7-5 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). From Lazarchick J. Sickle cell disease—RBC morphology—4. ASH Image Bank 2011; 2011-3961.

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 Hb 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 Hb SS or S β ⁰ (and in adults with Hb SC 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 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 Hb F 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), 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.

Table 7-3 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 deficiency.

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 Hb SS, despite the smaller red blood cells, lower MCH concentrations, and higher levels of Hb F and Hb A₂ associated with this genotype. Patients with Hb SC 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 Hb C does not enter into the deoxyhemoglobin S polymer, patients with Hb SC have symptoms, whereas those with sickle cell trait (AS) do not. This is thought to be caused by two important consequences of the presence of Hb C: the Hb S content in Hb SC is 10%-15% higher than that seen in sickle trait (Hb S 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 Hb C 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 7-6). Finally, in Hb SC 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 Hb S β^+ thalassemia have less severe anemia and pain than patients with Hb S β^0 thalassemia. This is the

result of smaller cells, lower MCHC, increased content of Hb F and Hb A₂, and, most important, the presence of significant amounts (10%-30%) of Hb A that interferes with polymerization.

Treatment

Treatment of sickle cell disease includes general preventive and supportive measures, as well as treatment of specific complications. The updated National Institutes of Health monograph entitled “*The Management of Sickle Cell Disease*” (Publication No. 02-2117) 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 13-valent pneumococcal conjugate vaccine (PCV-13), the 4-valent meningococcal conjugate vaccine (MCV-4), the 23-valent pneumococcal polysaccharide vaccine (PPV-23), and vaccines against *Haemophilus influenzae* and hepatitis B virus, in addition to twice-daily penicillin prophylaxis at least until the age of 5 years. Vaccinations against influenza on an annual basis and the vaccine against *pneumococcus* 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. Screening transcranial Doppler (TCD) ultrasonography to determine risk of overt stroke should be performed at least yearly for children of age 2-16 years with Hb SS or S β^0 thalassemia (see further discussion of TCD in the sections “Central nervous system disease” and “RBC transfusion”). 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 for 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

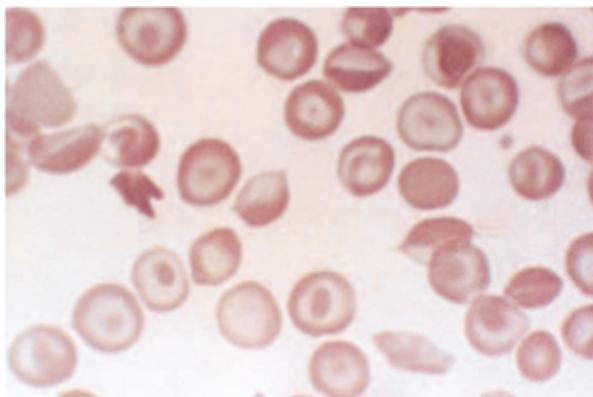


Figure 7-6 Sickle-hemoglobin C disease. This peripheral blood film shows no irreversibly sickled cells, as expected for hemoglobin SC, but shows instead a large number of target cells and several dense, contracted, and folded cells containing aggregated and polymerized hemoglobin.

Table 7-4 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 Hb concentration to 10 g/dL is as effective as exchange transfusion to reduce Hb S 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 transcranial Doppler (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 9-18 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 ($N=0$).

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 (NSAIDs), 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 (PCA) pumps or with administration at fixed intervals. Constant infusion 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 depres-

sion 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. Typically there are abnormalities on the chest examination. As the nonspecific term implies, various insults or triggers can lead to acute chest syndrome. Young age, low Hb F, high steady-state Hb, and elevated white blood cell count in steady state have been identified as risk factors. In a multicenter prospective study, bacterial 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 1.0-1.5 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 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%-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 (moya moya) near the site of previous infarction. Suspicion of a neurologic event requires emergent imaging with computed tomography (CT) to assess for hemorrhage followed by magnetic resonance imaging (MRI). The acute management of overt stroke includes transfusion, usually by an exchange technique, to reduce the Hb S percentage to <30%. Chronic transfusion therapy to maintain the Hb S <30% decreases the chance of recurrent overt stroke but does not eliminate it. After 3-5 years of such transfusions and no recurrent neurologic events, some physicians "liberalize" the transfusion regimen to maintain the Hb S <50%. The optimal duration of transfusions is not known, and they often are continued indefinitely. A randomized controlled trial (the SWiTCH study) of continued chronic transfusions versus 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 Hb SS at high risk of primary overt stroke. A randomized controlled trial of prophylactic transfusions versus observation for children with abnormal TCD

velocities showed a reduced risk of the first stroke in patients receiving transfusions (the STOP study). The use of hydroxyurea currently is being explored as means of primary stroke prevention in a phase III multicenter randomized controlled trial for children with abnormal TCD velocities (the TWiTCH study).

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. 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, acute toxemia). Close follow-up is indicated postpartum when the patient is still at high risk for complications. The option of contraception with an intrauterine device, subcutaneous implant, oral low-dose estrogen pills, 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 of 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 two 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 Hb S to <30% and increasing the Hb level to 9-10 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 Hb SS at high risk of primary overt stroke, which can be prevented by chronic transfusion therapy. Transfusion also has 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 Hb level of 9–10 g/dL with the Hb S under 30%–50%. It is important to avoid the hyperviscosity associated with Hb levels >11–12 g/dL in the presence of 30% or more Hb S. Patients with Hb SC requiring transfusion pose special challenges, with the need to avoid hyperviscosity usually necessitating exchange transfusion to ensure the Hb concentration does not exceed 11–12 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 a multicenter study, simple transfusion to a total Hb level of 10 g/dL afforded protection equal to partial exchange and was associated with fewer complications. 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. 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 will become alloimmunized to RBC antigens (especially E, C, and Kell), and this risk increases with increasing exposure. Alloimmunization predisposes patients to delayed transfusion reactions. Severe painful crises with a decrease in the Hb 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, two other disease-modifying treatments currently are available: (i) hydroxyurea, which is ameliorative; and (ii) hematopoietic stem cell transplantation, which is curative. On the basis of knowledge that patients with high Hb F levels have less severe disease, many investigators tested a variety of experimental strategies for

pharmacologic induction of Hb 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 Hb SS patients (the 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 Hb F 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 9 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. Many clinicians now use hydroxyurea more liberally, however, given the safety of hydroxyurea and that Hb SS is a morbid condition even when the classical indications for hydroxyurea therapy are not present. 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. Hematopoietic stem cell transplantation has been used primarily for children with stroke or other severe disease manifestations, with an event-free survival rate of >80%. In most centers, few patients meet the usual eligibility criteria, which includes an HLA-matched sibling donor. Alternative donor sources such as umbilical cord blood are now being used. As novel approaches, such as nonmyeloablative conditioning regimens, undergo further development, stem cell transplantation for patients with sickle cell disease could be greatly expanded.

Clinical case (continued)

The case in this section describes a patient with sickle cell anemia who has experienced pain episodes but no other 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. It often is precipitated by a severe acute pain crisis and is thought to be secondary to widespread intravascular sickling 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 is a consideration 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.

Hemoglobin E

Hb E is one of the two most prevalent Hb mutants worldwide. It has become more common in the United States during the past 20–30 years as a result of immigration. Hb E is found with highest frequency in Southeast Asians and has its highest prevalence in Burma and Thailand, where the gene frequency may approach 30%. The gene frequency is also high in Laos, Cambodia, and Vietnam. The structural change is a substitution of glutamic acid by lysine at the 26th position of the β -globin chain (Figure 7-4). 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 direct translation. Hb E is also slightly unstable in the face of oxidant stress. Hb E is sometimes referred to as a “thalassemic hemoglobinopathy.”

Patients heterozygous for Hb E (Hb E trait) have no anemia, mild microcytosis (MCV approximately 71–75 fL in

adults and as low as 65 fL in children), target cells, and 30%–35% Hb E. The Hb E concentration will be lower with the coinheritance of α thalassemia. Homozygotes (Hb E disease) may have mild anemia, microcytosis (MCV approximately 65–69 fL in adults and 55–65 fL in children), and substantial numbers of target cells. The compound heterozygous state, Hb E- β thalassemia, is now one of the more common forms of thalassemia intermedia (and thalassemia major) in the United States. Hb E- β^0 thalassemia is associated with a mostly Hb E electrophoretic pattern, with increased amounts of Hb F and Hb A₂. The electrophoretic pattern in Hb E- β^+ thalassemia is similar except for the presence of approximately 15% Hb A. Hb E comigrates with Hb C and Hb A₂ on cellulose acetate electrophoresis and isoelectric focusing.

Patients with Hb E disease are usually asymptomatic and do not require specific therapy. However, patients who coinherit Hb E and β -thalassemia, especially those with Hb E- β^0 , may have significant anemia. Some need intermittent or chronic RBC transfusions, and some may benefit from splenectomy.

Hemoglobin C

Hb C is the third most common mutant Hb, after Hb S and Hb E. The Hb C mutation arose in West Africa. The prevalence in African Americans is 2%–3%. The Hb mutant results from the substitution of lysine for glutamic acid as the sixth amino acid of β -globin, the consequence of a single nucleotide substitution (GAG/AAG) in the sixth codon (Figure 7-4). The resultant positive-to-negative charge difference on the surface of the Hb C tetramer results in a molecule with decreased solubility of both the oxy and deoxy forms that may undergo intraerythrocytic aggregation and crystal formation. Hb C 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 Hb CC and patients with C 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 (Hb C trait) are clinically normal. The coinheritance of Hb S and Hb C results in a form of sickle cell disease, Hb SC (see the section “Sickle Cell Disease”).

Laboratory studies in Hb CC show a hemolytic anemia and slightly elevated reticulocyte counts. The MCHC is elevated because of the effect of Hb C on cellular hydration. The MCV generally is reduced. The blood smear shows prominent target cells. RBCs containing Hb crystals also may be seen on the blood smear, particularly in patients who have had splenectomy. Individuals with Hb C trait have normal Hb levels but may have target cells on the peripheral smear.

Confirmation of the diagnosis requires identification of Hb C, which comigrates with Hb A₂, Hb E, and Hb O^{Arab} on cellulose acetate and isoelectric focusing. Thus, Hb C must be distinguished by citrate gel electrophoresis or HPLC.

Specific treatment for patients with Hb CC is not available or necessary.

Hemoglobin D

Hb D usually is diagnosed incidentally. Hb D^{Punjab} (also called Hb D^{Los Angeles}) results from the substitution of glutamine for glutamic acid at the 121st position of the β-chain (Figure 7-4). This mutant has a prevalence of approximately 3% in the Northwest Punjab region of India but also is encountered in other parts of the world. Patients with homozygous (Hb DD) or heterozygous (Hb AD) do not have hemolysis. The major clinical relevance of Hb D is with compound heterozygous inheritance with Hb S, resulting in a form of sickle cell disease, perhaps as a result of the low-affinity Hb D promoting Hb deoxygenation. The diagnosis of Hb AD (D trait) or DD is made by Hb electrophoresis. Hb S and Hb D 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 mutant hemoglobins that can have significant consequences when coinherit with hemoglobin S.
- Homozygosity for hemoglobin E (EE) is a mild condition, but compound heterozygosity for Hb E and β-thalassemia can be a clinically significant thalassemia syndrome.

Unstable Hb

Unstable Hb mutants are inherited in an autosomal dominant pattern, and affected individuals are usually heterozygotes. Unstable Hbs constitute one of the largest groups of Hb variants, although individually, each is rare. In Hb Köln, the most prevalent unstable Hb, the β₉₈ Val/Met substitution destabilizes the heme pocket. In Hb Zurich, the β₆₃ His/Arg also disrupts the heme pocket. Other mechanisms that destabilize Hb include (i) alteration of the α₁β₁ interface region (eg, Hb Tacoma, β₃₀ Arg/Ser); (ii) distortion of the helical configuration of structurally important regions (eg, Hb Bibba, α₁₃₆ Leu/Pro); and (iii) introduction of the interior polar amino acid (eg, Hb Bristol, β₆₇ Val/Asp). Unstable γ-chain variants (eg, Hb Poole, γ₁₃₀ Trp/Gly) can cause transient hemolytic anemia in the neonate that will spontaneously resolve.

These abnormal Hbs precipitate spontaneously or with oxidative stress. Precipitated Hb inclusions (Heinz bodies) 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 Hb level may be normal or decreased. Hypochromia of the RBCs (resulting from loss of Hb due to denaturation and subsequent pitting), “bite cells,” and basophilic stippling may occur. The evaluation includes Hb electrophoresis (which is often normal), crystal violet Heinz body staining, and the isopropanol stability test. The isopropanol test may be falsely positive in the neonate due to high fetal Hb 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.

Certain Hb M mutations also render the molecule unstable, producing a clinical picture of both cyanosis (methemoglobinemia without desaturation) and hemolysis. Some unstable Hbs also may have altered oxygen affinity, which could exacerbate (decreased oxygen affinity) or ameliorate (increased oxygen affinity) the degree of anemia.

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 only for intermittent jaundice and a cholecystectomy for gallstones at age 22 years. She takes no medications. A cousin and an aunt have also had anemia and jaundice. Her examination is significant for mild splenomegaly. Prior laboratory data reveal hematocrit values between 29% and 33%. Today's hematocrit is 27%, MCV 98 fL, MCHC 38 g/dL. Corrected reticulocyte count is 7%. Review of the peripheral blood smear reveals numerous spherocytes.

Hereditary spherocytosis (HS), hereditary elliptocytosis (HE), and hereditary pyropoikilocytosis (HPP) are a heterogeneous group of disorders with a wide spectrum of clinical manifestations. This group of disorders is characterized by abnormal shape and flexibility of RBCs because of a deficiency or dysfunction of one or more of the membrane proteins, which leads to shortened RBC survival (hemolysis).

Multiple genetic abnormalities, including deletions, point mutations, and defective mRNA processing, have been identified as underlying causes. The HS syndromes generally are due to private mutations unique to each kindred. In contrast, some HE syndromes are due to specific mutations in individuals from similar locales (eg, Melanesian elliptocytosis), suggesting a founder effect.

RBC membrane protein composition and assembly

The RBC membrane consists of a phospholipid-cholesterol lipid bilayer intercalated by integral membrane proteins such as band 3 (the anion transport channel) and the glycophorins (Figure 7-7). 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 fiber-like 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 two-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 are 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 two 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. 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. Because the distal ends of spectrin bind to actin, with the aid

of protein 4.1 and other minor proteins (Figure 7-7), 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 is caused by defects in horizontal interactions.

Hereditary spherocytosis

HS is common in individuals of Northern European descent with an occurrence of approximately 1 in 2,000. 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. HS is characterized by spherocytic, osmotically fragile RBCs and is both clinically and genetically heterogeneous.

Pathophysiology

The pathophysiology of HS generally involves aberrant interactions between the skeleton and the overlying lipid bilayer (“vertical interactions”). A common epiphénomène 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

Figure 7-7 The RBC membrane. A model of the RBC membrane is shown in which the relative positions of the various proteins is correct, but the proteins and lipids are not drawn to scale. From Handin RI, Lux RJH, Stossel T. *Blood: Principles and Practice of Hematology*. Philadelphia, PA: Lippincott-Raven; 1995.

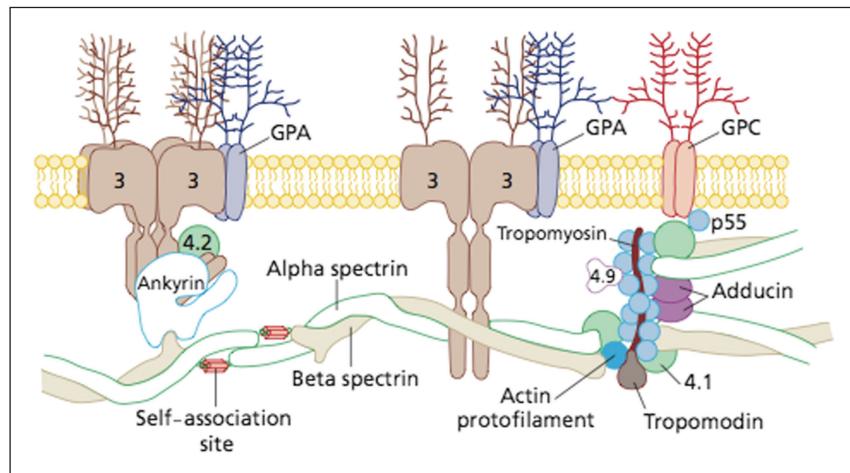


Table 7-5 Defects of red blood cell membrane proteins in hereditary spherocytosis, elliptocytosis, and pyropoikilocytosis.

Class of defect	Heredity spherocytosis	Heredity elliptocytosis and pyropoikilocytosis
Protein deficiency	Spectrin Ankyrin* Band 3 Protein 4.2	Spectrin [†] Protein 4.1 Glycophorin C
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 the 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.

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 7-5). A deficiency or defect of the ankyrin molecule represents the most common cause of dominant HS. In 30%-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 Hb levels (compensated hemolysis) to severe hemolysis and anemia. Patients with mild HS have a relatively uneventful course, although some may develop pigmented (bilirubinate) 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. 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 7-8(a)]. 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. The osmotic fragility test using increasingly hypotonic saline solutions will support 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. Osmotic fragility testing is a test for spherocytes of any cause, not a specific test for HS. Related to standard osmotic fragility testing is the newer osmotic gradient ektacytometry, which measures deformability of whole RBCs measured as a

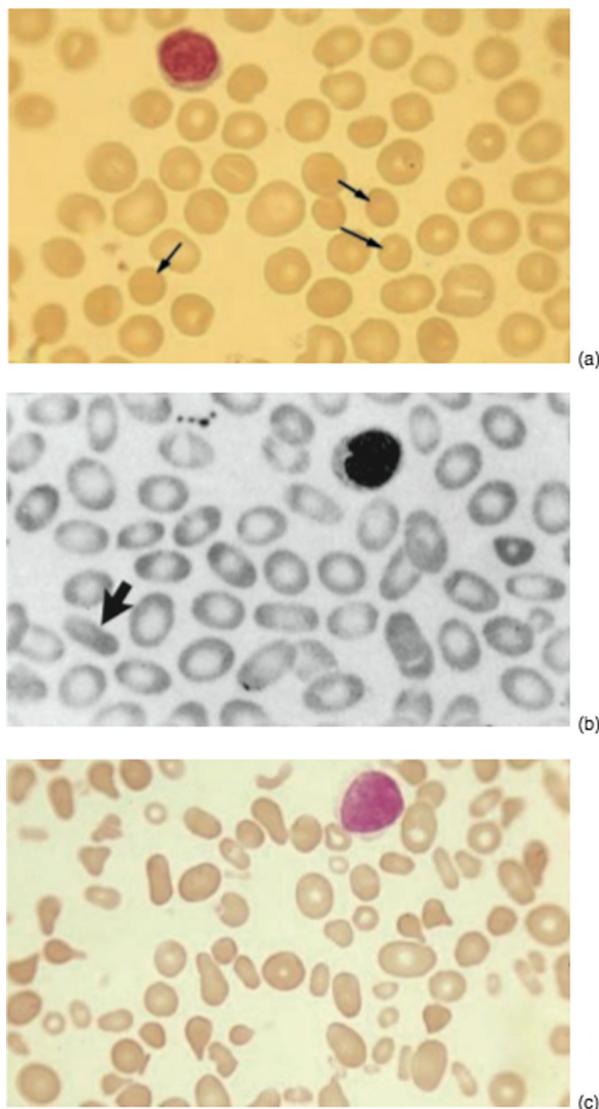


Figure 7-8 Peripheral blood findings in inherited disorders of the red cell membrane: (a) numerous spherocytes (arrows), (b) numerous elliptocytes and a rod-shaped cell (arrow), and (c) marked poikilocytosis.

function of osmolality using a laser-diffraction viscometer. Ektacytometry appears to be more sensitive than standard osmotic fragility testing, and it can help differentiate HS from HE, HPP, and stomatocytosis. A different test for HS and related cytoskeleton-associated hemolytic anemias is the eosin-5-maleimide (EMA) binding assay. EMA binds to band 3 on RBCs, and a reduction in binding, measured by fluorescence intensity, corresponds to a quantitative reduction in erythrocyte band 3, consistent with HS. The EMA-binding test has a higher predictive value for HS than standard osmotic fragility testing. Review of the complete blood count, reticulocyte count, and peripheral smear from family members may prove helpful. The differential diagnosis

for spherocytes and increased osmotic fragility includes autoimmune hemolytic anemia, so 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.

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. 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. In rare patients with HS and severe hemolysis, the procedure markedly diminishes the hemolytic rate but may not fully correct the anemia. Controlled 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 surgery for patients with symptomatic hemolytic anemia or its complications, particularly gallstones. 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 postsplenectomy 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 (4–6 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.

Clinical case (continued)

The patient presented in this section should be suspected of having HS. 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, and spherocytes on peripheral smear all support the diagnosis. The diagnosis should be confirmed by demonstrating increased osmotic fragility of RBCs, especially if the spherocytosis is not obvious on the peripheral blood film, and by a negative DAT. 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.
- The diagnosis of HS can be supported by the osmotic fragility test, the sensitivity of which is increased with incubation at 37°C. Osmotic gradient ektacytometry and the EMA-binding assay are newer, often more sensitive, tests for membranopathies like HS and HE.
- Splenectomy decreases hemolysis and reduces gallstone formation, but it should be reserved for symptomatic or severe 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 than in HS. Three distinct subtypes are distinguished: (i) common HE, characterized by biconcave elliptocytes and, in more severe forms, rod-shaped cells, poikilocytes, and fragments [Figure 7-8(b)]; (ii) spherocytic HE, a phenotypic hybrid between HE and HS; and (iii) Southeast Asian ovalocytosis with unique spoon-shaped erythrocyte morphology. In most cases, the inheritance of HE is autosomal dominant. The exception is HPP, a rare and severe variant of common HE that is recessively inherited [Figure 7-8(c)]. The clinical expression of common HE, the most prevalent form of ellip-

tocytosis, is highly variable, ranging from an asymptomatic carrier state to a severe hemolytic disease 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 7-5). 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 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, 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 from the previous conditions. Whereas most patients with common HE 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. The most severe form of elliptocytosis, HPP, typically is inherited recessively and is characterized by a striking micropoikilocytosis 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-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 in the extract can be detected by electrophoresis of extracts under nondenaturing conditions.

Treatment is not necessary for most individuals with common HE. 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 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 rare condition with apparent coinheritance of spectrin defects leading to markedly abnormal red cells characterized by increased thermal instability.

Other RBC membrane disorders

Stomatocytosis

Stomatocytes have a wide transverse slit or stoma toward the center of the RBC (Figure 7-9). 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 cation permeability and volume, which is either increased (hence, the designation hydrocytosis), decreased (xerocytosis), or near normal. Of significant clinical importance is the recognition that patients with hereditary stomatocytosis have an increased risk of developing thrombotic events after splenectomy. 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.

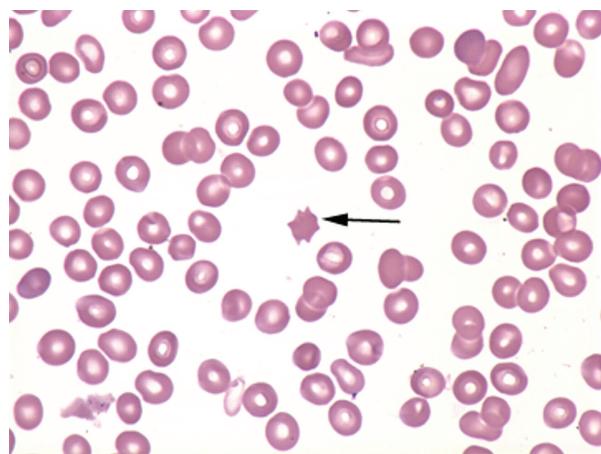


Figure 7-9 Stomatocytes.

Acanthocytosis

Spur cells, or acanthocytes (from the Greek *acantha*, or thorn; Figure 7-10), 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 two-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.

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.

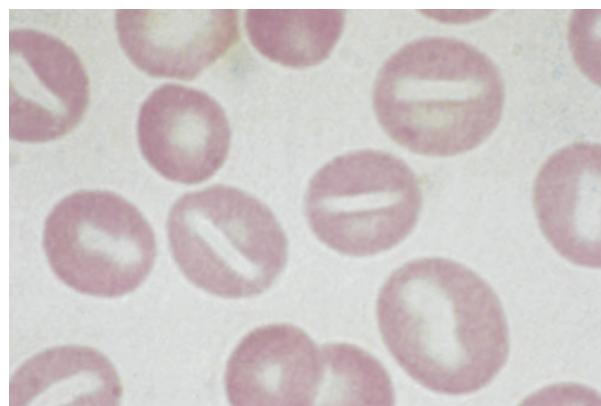


Figure 7-10 Acanthocytes.

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.

Acanthocytes also have been described in patients with the McLeod phenotype, a condition in which the erythrocytes have reduced surface Kell antigen because the Kx protein needed for its expression is absent. The Kx antigenic protein is encoded by the X chromosome, so males are affected with mild compensated hemolysis, whereas asymptomatic carrier females may be identified by flow cytometric analysis of Kell blood group antigen expression.

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 autosomal 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. Stomatocytes and occasional spherocytes are the result of this dehydration and can be identified 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 is referred for evaluation of anemia. His past history is significant for an

Clinical case (continued)

episode of hemolytic uremic syndrome (HUS) that led to renal failure 2 years prior to referral. He had no further relapses of HUS or thrombotic thrombocytopenic purpura (TTP). His posttransplant course has been unremarkable with good graft function and no rejection. When he left the hospital, his hematocrit was 31%. His discharge medications included prednisone, cyclosporine, trimethoprim/sulfamethoxazole, and acyclovir. He complains of increasing fatigue and dyspnea over the 10 days since discharge. 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 hematocrit is 20%, corrected reticulocyte count 10%, LDH 1,543 U/L. Serum creatinine is 1.8 mg/dL and the platelet count is 302,000 /mm³, 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 two principal pathways of glucose catabolism: the glycolytic pathway and the hexose-monophosphate shunt. The three major functions of the products of glucose catabolism in the erythrocyte are (i) maintenance of protein integrity, cellular deformability, and RBC shape; (ii) preservation of Hb iron in the ferrous form; and (iii) modulation of the oxygen affinity of Hb. These functions are served by the regulation of appropriate production of five specific molecules: ATP, reduced glutathione, reduced NADH, reduced NADPH, and 2,3-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 five essential RBC substrates: glucose, glutathione, NAD, NAD phosphate (NADP), and adenosine diphosphate (ADP).

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 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 Hb oxygen affinity, thus facilitating the transfer of oxygen from Hb to tissue-binding sites. The major function of the hexose-monophosphate shunt is preservation and regeneration of reduced glutathione, which protects Hb and other intracellular and membrane proteins from oxidant injury.

Abnormalities of the glycolytic pathway

Defects in the glycolytic pathway lead to a decrease in the production of ATP or a change in the concentration of 2,3-BPG.

Deficiencies of erythrocyte hexokinase, glucose phosphate isomerase, phosphofructokinase, and pyruvate kinase (PK) all lead to a decrease in ATP concentration. Although genetic disorders involving nearly all of the enzymes of the glycolytic pathway have been described, PK accounts for >80% of the clinically significant hemolytic anemias from defects in this pathway. With the exception of phosphoglycerate kinase deficiency, which is X-linked, all other glycolytic enzyme defects are autosomal recessive.

PK deficiency is the most common congenital nonspherocytic hemolytic anemia caused by a defect in glycolytic RBC metabolism. The syndrome is both genetically and clinically heterogeneous. PK deficiency has a worldwide distribution but is more common among those of northern and eastern European heritage. Severe cases can present either with neonatal jaundice or in early childhood with jaundice, splenomegaly, and failure to thrive. Alternatively, a mild presentation with fully compensated hemolytic anemia has been described. Osmotic fragility of the patient's RBCs is typically normal and may be helpful in differentiating this condition from HS. Several screening tests have been developed to diagnose PK deficiency, but often they lack sensitivity to diagnose specific PK variants. Reference laboratories can perform quantitative measurement of the erythrocyte enzyme level necessary to diagnose this condition accurately.

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 show small dense crenated cells (echinocytes or "prickle cells") (Figure 7-11). In the more 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

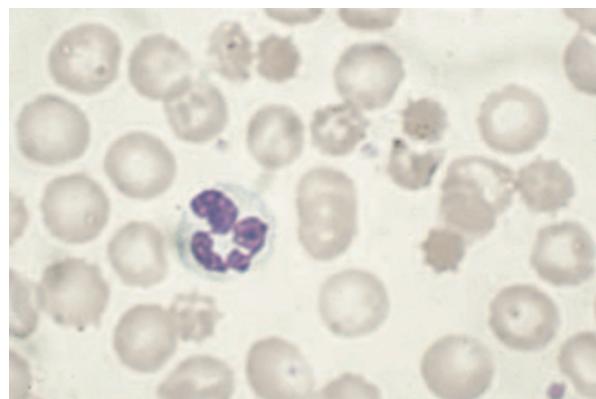


Figure 7-11 Pyruvate kinase deficiency. The peripheral blood film shows many small dense crenated cells (echinocytes).

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 Hb level. Splenectomy may be complicated by postoperative thromboembolic phenomena.

Abnormalities of the hexose-monophosphate shunt

G6PD deficiency is the most frequently encountered abnormality of RBC metabolism, affecting >200 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. 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 two clonal RBC populations, one normal and one 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 according to whether the defect leads to normal activity, moderately deficient activity, or severely deficient activity, and whether it is associated with hemolytic anemia. 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%-15% of African American males. This variant is an unstable enzyme, which results in a decrease in enzyme activity in aged RBCs. 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 the G6PD B variant, G6PD-Mediterranean.

Hemolysis in G6PD-deficient RBCs is due to a failure to generate adequate NADPH, leading to insufficient levels of reduced glutathione. This renders erythrocytes susceptible to oxidation of Hb by oxidant radicals, such as hydrogen peroxide. The resulting denatured Hb 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.

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 is sometimes the precipitating cause (Table 7-6). 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. Hemolysis triggered by exposure to naphthalene (moth balls) is now much less common in children. 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 Hb 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 population of G6PD sufficient (normal) cells coexists. 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.

Certain G6PD variants may result in a congenital non-spherocytic hemolytic anemia with persistent splenomegaly. Affected individuals are extremely susceptible to the oxidant stress associated with the drugs and disorders mentioned previously and also may exhibit severe hemolysis ("favism") after ingestion of fava beans. 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.

When hemolytic anemia occurs after the ingestion of an oxidant drug or in association with the clinical states leading to oxidant stress, G6PD deficiency should be considered. Significant anemia, hyperbilirubinemia, elevated plasma Hb, and hemoglobinuria may be due to brisk intravascular hemolysis. G6PD deficiency should be considered in an individual with evidence of chronic DAT-negative hemolysis. The peripheral blood smear reveals RBCs with the Hb confined to one side of the cells, with the remainder appearing as an Hb-free ghost (eccentrocytes) (Figure 7-12). The morphology previously has been described as bite or blister cells, interpreted as the result of removal of denatured Hb by the spleen;

Table 7-6 Agents that cause clinically significant hemolysis in patients with G6PD deficiency.

Acetanilide	Pentaquine
Dapsone	Phenylhydrazine
Dimercaptosuccinic acid	Phenazopyridine
Furazolidone	Primaquine
Glibenclamidine	Sulfacetamide
Isobutyl nitrite	Sulfamethoxazole
Methylene blue	Sulfanilamide
Nalidixic acid	Sulfapyridine
Naphthalene	Thiazolesulfone
Niridazole	Toluidine blue
Nitrofurantoin	Trinitrotoluene (TNT)
Pamaquine	Urate oxidase

Data from Beutler E. Glucose-6-phosphate dehydrogenase deficiency. In: Starbury JB, Wyngaarden JB, Frederickson DS, et al, eds. *The Metabolic Basis of Inherited Disease*. 5th ed. New York: McGraw-Hill, 1983. Updated from Beutler E. Glucose-6-phosphate dehydrogenase deficiency and other red cell enzyme abnormalities. In Beutler E, Lichtman MA, Coller BS, et al, eds. *Williams Hematology*. 6th ed. New York: McGraw-Hill, 2001.
G6PD = glucose-6-phosphate dehydrogenase.

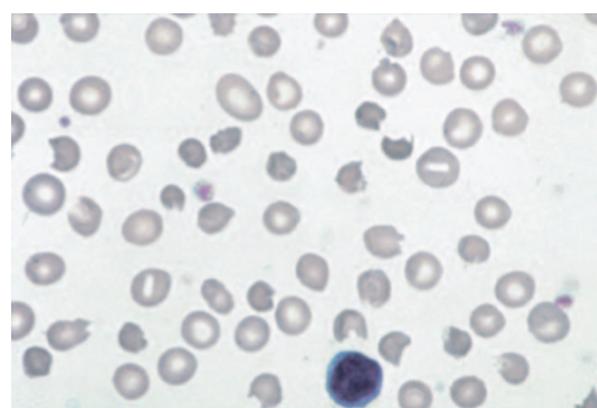


Figure 7-12 G6PD deficiency. The peripheral blood smear shows several red cells with the hemoglobin confined to one side of the cells with the remainder appearing as a hemoglobin-free ghost (eccentrocytes).

however, it appears that the accumulated oxidized Hb 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 will raise 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. Although defects of other hexose-monophosphate shunt enzymes (eg, phosphogluconate dehydrogenase, glutathione reductase) are rare, they should be considered in cases of oxidant-induced hemolysis in which G6PD levels are normal.

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. 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.

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 will 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, however, a trial of continuation is reasonable as hemolysis often is compensated in the G6PD A⁻ variant even if drug administration is continued.

Key points

- The glycolytic pathway provides the erythrocyte with the ATP necessary for maintenance of membrane integrity, preservation of ferrous hemoglobin, and oxygen affinity. Additional products of the pathway are NADH for methemoglobin reduction and 2,3-BPG for regulating the oxygen affinity of hemoglobin.

Key points (continued)

- Glucose metabolism through the hexose monophosphate shunt produces NADPH to maintain the antioxidative activity of the red cell.
- 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. The echinocyte is the characteristic abnormality observed on the peripheral blood smear.
- The most common enzyme deficiency is G6PD with >300 genetic variants. Oxidant stress in the presence of deficient G6PD activity results in hemolysis with the generation of blister cells and bite cells (eccentrocytes).
- In the G6PD A⁻ deficiency, a quantitative measurement of the enzyme levels should be delayed until after the acute hemolytic episode. In the G6PD B variant (eg, G6PD-Mediterranean), levels are low in red cells of all ages.
- Defects of purine and pyrimidine metabolism are infrequent. The peripheral blood smear in pyrimidine-5'-nucleotidase deficiency shows red cells with coarse basophilic stippling.

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 are mildly cyanotic appearing. Laboratory data are significant for a spun hematocrit of 24% and an MCV of 143 fL. LDH is elevated at 2,321 U/L, indirect bilirubin at 2.1 mg/dL, and reticulocyte count at 13%. The peripheral blood smear shows agglutinated RBCs. The blood bank reports a direct Coombs test positive for complement (3+) but negative for immunoglobulin G (IgG). Serum protein electrophoresis reveals a monoclonal IgM. 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%-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 random 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 three different mechanisms. Some drugs induce

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 7-7.

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

Table 7-7 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, Hodgkin disease, 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 ingestion of certain drugs (eg, α-methyldopa)
 - 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 clonal B-lymphocyte proliferation)
 - 2. Secondary cold agglutinin hemolytic anemia
 - a. Postinfectious (eg, *Mycoplasma pneumoniae* or infectious mononucleosis)
 - b. Associated with malignant B-cell lymphoproliferative disorder
 - B. Mediated by cold hemolysins
 - 1. Idiopathic (primary) paroxysmal cold hemoglobinuria
 - 2. Secondary
 - a. Donath-Landsteiner hemolytic anemia, usually associated with an acute viral syndrome in children (relatively common)
 - 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
 - D. Nonimmunologic protein adsorption (probably does not cause hemolysis)
-

may or may not fix complement, but they typically do not cause direct agglutination of RBCs because of their small size. 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, 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 C3b-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 is typically seen in the elderly, almost always associated with B-cell lymphoproliferative disorders; 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, may cause significant intravascular lysis of RBCs as a result of their ability to fix complement. Donath-Landsteiner hemolytic anemia frequently was associated with congenital syphilis when that disease was common. Now, it is almost always idiopathic. Donath-Landsteiner hemolytic anemia accounts for almost one-third 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 7-8). Three basic mechanisms of drug-induced immune RBC injury are recognized. A fourth mechanism may lead to nonimmunologic deposition of multiple serum proteins, including immunoglobulins, albumin, fibrinogen, and others, on RBCs, but RBC injury does not occur. The

Table 7-8 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	Probenecid
Diclofenac	Quinine
Diethylstilbestrol	Quinidine
Doxepin	Rifampicin
Etodolac	Stibophen
Hydrocortisone	Thiopental
Metformin	Tolmetin
Autoantibody Mechanism	
Cephalosporins	Lenalidomide
Cianidanol	Mefenamic acid
Cladribine	α-Methyldopa
Diclofenac	Nomifensine
l-Dopa	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	Mephénytoïn
Carboplatin	Nalidixic acid
Chlorpromazine	Omeprazole
Efavirenz	Phenacetin
Erythromycin	Streptomycin
Fluorouracil	Sulindac
Ibuprofen	Temafloxacin
Insecticides	Thiazides
Isoniazid	Triamterene

Table 7-9 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 three-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

Modified from Packman CH. Hemolytic anemia resulting from immune injury. In: *Williams Hematology*. 8th ed. New York, NY: McGraw-Hill; 2010. Ig = immunoglobulin.

mechanisms of drug-induced immune-hemolytic anemia and positive DATs are summarized in Table 7-9. Second- and third-generation cephalosporins account for about 88% 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 (IAT) 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 7–10 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 three 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, cisplatin and carboplatin, develop positive antiglobulin reactions because of nonspecific adsorption of plasma proteins to their RBC membranes. This process may occur within 1-2 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 cross-match 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 7-13). 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.

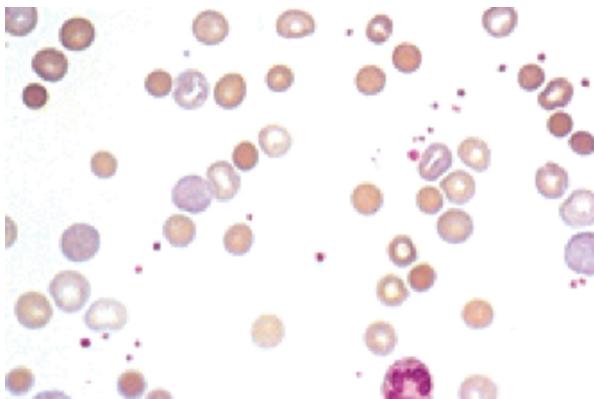


Figure 7-13 Warm-antibody autoimmune hemolytic anemia. Note the small round spherocytes and the large, gray polychromatophilic erythrocytes.

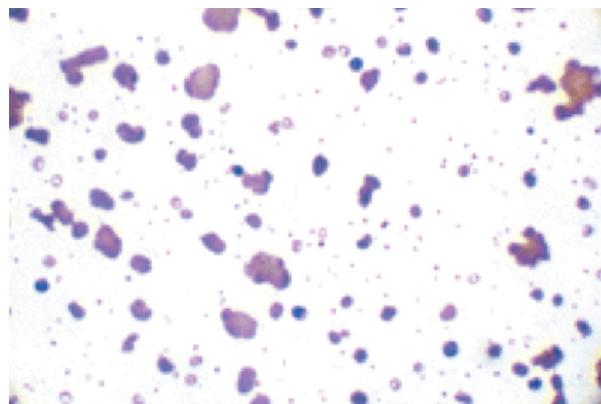


Figure 7-14 Cold agglutinin disease.

Patients with idiopathic or primary cold agglutinin disease 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 7-14). Occasionally spurious marked elevations in the MCV and MCHC measurements and decrease in the RBC count are observed due to simultaneous passage of two or three 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 (Coombs test) 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 fragments of C3, is approximately 200-500 antibody molecules per cell. However, <100 molecules of IgG per cell may significantly shorten RBC survival in vivo. IgM cold agglutinins usually are removed from RBCs during washing and usually are not detected. IgA usually is not detected by most commercial reagents. When monospecific anti-IgG and anti-C3 reagents are used, 30%-40% of patients with AHA will have only IgG on their RBCs; a slightly larger number will have both IgG and C3; and only approximately 10% will have C3 alone. The major reaction patterns of the DAT and their differential diagnosis are summarized in Table 7-10.

The strength of the direct Coombs test has poor clinical correlation with severity of hemolysis among patients, but in a given patient over time, the degree of hemolysis correlates fairly well with the current strength of the antiglobulin reaction. In the rare case of direct Coombs test-negative hemolytic anemia suspected of having an immune etiology, the diagnosis sometimes can be confirmed by using more sensi-

Table 7-10 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)

IgG = immunoglobulin G.

tive assays for RBC-bound immunoglobulin, such as an enzyme-linked immunoadsorbent assay (ELISA) or radiolabeled anti-immunoglobulin. Specific assays for cell-bound IgA also may be worthwhile. In cold agglutinin disease, 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 paroxysmal cold hemoglobinuria 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. 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 reaction. The difficult technical issue relates to detection of RBC alloantibodies masked by the presence of the autoantibody.

Clinicians and blood bank physicians no longer speak of "least incompatible" blood for transfusion because all units will be 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.

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.

In AHA, therapy is aimed at decreasing the production of autoantibody 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 60–100 mg/d (or 1 mg/kg/d), the dose should be decreased by 10 mg/d each week until a dose of 30 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 20–40 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 approximately 20% 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 2–3 weeks at prednisone doses of 1 mg/kg, if remission cannot be maintained on low doses of prednisone, or if the patient has intolerable adverse effects or contraindications to glucocorticoids. Removing the spleen 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 will have complete or partial remission with splenectomy, but relapses are common.

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) monoclonal anti-CD20 (rituximab) has been useful in refractory cases. Adults and children respond equally well with response rates ranging from 40% to 100%. 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 TTP.

For patients with idiopathic cold agglutinin disease, maintaining a warm environment may be all that is needed to avoid symptomatic anemia. Cold agglutinin disease usually does not respond to glucocorticoids. Recently, rituximab has demonstrated efficacy in treating cold agglutinin disease, with response rates approaching 50%. Chlorambucil and cyclophosphamide have been beneficial in selected cases. Chemotherapy is indicated if the disorder is associated with a lymphoproliferative disorder. Splenectomy usually is not indicated because cells usually 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. Recently, a combination of fludarabine and rituximab has been used with success, but toxicity is a concern.

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 antibody hemolytic anemia, 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 refractory disease that warrants consideration of other pharmacologic agents or splenectomy.

Clinical case (continued)

The patient presented in this section has cold agglutinin disease, likely secondary to underlying lymphoma. Automated techniques reveal the red cell count is artifactually 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 will minimize agglutination. The direct antiglobulin test 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.
- Cold agglutinin disease 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 autoimmune hemolytic anemia are typically indistinguishable from other causes of hemolysis.
- The direct antiglobulin test is the primary tool for diagnosing autoimmune hemolytic anemia. It is rarely positive in healthy individuals and may be negative in autoimmune hemolytic anemia.
- Warm-antibody-mediated autoimmune hemolytic anemia is treated with glucocorticoids, other immunosuppressive agents such as rituximab, and splenectomy.
- Avoidance of cold environments may be sufficient to avoid complications of cold agglutinin disease. Chemotherapy and rituximab have a role, and plasmapheresis occasionally can be helpful in the acute and temporary management of symptomatic cases by physically removing the antibody.
- Immune-mediated hemolytic anemia is uncommon in children. Most cases are acute and transient, following viral infection.
- Transfusion therapy can be difficult in patients with autoimmune hemolytic anemia. 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 dangerous alloantibodies. No patient with AHA should be allowed to die 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. Workup 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 hematocrit of 32% with a corrected reticulocyte count of 8%. White count and platelet count are slightly depressed. Indirect bilirubin is elevated at 4 mg/dL, 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 are hypocellular and reveal findings concerning for early myelodysplasia.

Paroxysmal nocturnal hemoglobinuria (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

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. 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.

The biochemical basis of complement sensitivity was initially elusive. Early on, PNH blood cells were found to be deficient in leukocyte alkaline phosphatase and erythrocyte acetylcholinesterase. Neither of these deficiencies, however, explained the complement sensitivity or the clinical manifestations in PNH. Subsequently, two complement regulatory proteins, CD55 (decay accelerating factor [DAF]) and CD59 (homologous restriction factor or membrane inhibitor of reactive lysis [MIRL]), also were found to be missing from PNH blood cells, helping to explain the unusual sensitivity of RBCs to the hemolytic action of complement. Of these, CD59, whose action is to inhibit the terminal complement sequence leading to hemolysis, seems to be the most important.

The approximately 20 proteins missing from the hematopoietic cells in PNH are all attached to the membrane by a glycosylphosphatidylinositol (GPI) anchor. Defective synthesis of the GPI anchor is due to somatic mutations in the pig-A gene in hematopoietic stem cells. Whereas a pig-A gene mutation appears to be necessary for the development of PNH and its clinical manifestations, it is not sufficient because pig-A 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 pig-A mutations.

A multistep process seems necessary for PNH to develop. It is thought that in aplastic anemia and likely in PNH that 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: (i) urokinase plasminogen activator receptor, the lack of which may decrease local fibrinolysis; and (ii) 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. The drug does not seem to alter the defect in hematopoiesis. Thus, although deficient 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 latter 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 (DAF) and CD59 (MIRL). These 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.

A new assay is being used increasingly to detect GPI-deficient blood cells. The fluorescein-labeled aerolysin (FLAER) assay exploits a property of aerolysin, the principle virulence factor of the bacterium *Aeromonas hydrophila*, which binds selectively with high affinity to the GPI anchor of most cell lineages. FLAER is most useful to assay the GPI anchor on granulocytes because aerolysin binds weakly to glycophorin on RBCs.

Clinical manifestations

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. 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 Hb to scavenge nitric oxide. These include esophageal spasm, male erectile dysfunction, renal insufficiency, thrombosis, and pulmonary hypertension.

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 or myelodysplastic or myeloproliferative disorders 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.

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

The clinical manifestations of PNH are highly variable among patients. For patients with PNH clones numbering <10%, no clinical intervention is needed. Because expansion of the clone may occur, however, the size of the clone should

be monitored every 6–12 months. Anemia is often the dominant issue in PNH. Glucocorticoids can reduce complement activation and decrease the hemolysis; however, high doses are frequently necessary, and every-other-day administration has been recommended to reduce the adverse effects. Iron may be required to replace the large urinary losses seen in PNH. Folate supplementation usually is recommended as well. Erythropoietin (10,000–20,000 U three times weekly) may be helpful for patients with inadequate reticulocyte responses. Transfusion may be necessary when these measures fail to maintain adequate Hb levels.

Eculizumab is a humanized monoclonal antibody that was engineered to reduce its immunogenicity; importantly, it is unable to bind Fc receptors on cells or to activate complement. It binds to C5 and blocks the terminal complement sequence. The U.S. Food and Drug Administration approved its use in PNH to treat hemolysis based on efficacy in two phase III clinical trials. Eculizumab reduces intravascular but not extravascular hemolysis, eliminates or reduces transfusion requirement in almost all patients, improves quality of life, improves pulmonary hypertension, and decreases the risk of thrombosis. It does not treat the marrow failure. It must be used indefinitely because it does not treat the underlying cause of PNH.

Although eculizumab generally is 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 3–5 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.

Allogeneic hematopoietic stem cell transplantation is the only known cure for PNH. Because of the high risk for serious complications including death, however, such treatment should be reserved for patients with severe pancytopenia 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.

Thrombosis is the leading cause of death in PNH patients. Thrombosis 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, however, prophylactic anticoagulation is not indicated in these patients based on the current state of knowledge. The bigger question concerns prophylaxis in patients who do not require eculizumab; in general, lacking a randomized trial, it is probably not indicated until further studies are available. 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 is a pregnancy category C pharmaceutical; however, there are recent case reports of its apparent safe use in pregnancy. Also, patients with PNH undergoing surgery should receive prophylactic anticoagulation in the perioperative period. The recommended duration of either prophylactic or therapeutic anticoagulation has not been established.

Prognosis

The median survival for PNH is 10–15 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.

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 CD55 and CD59 on granulocytes, revealing a population of cells with absence of GPI-linked proteins. Treatment is aimed at the major clinical presentation. Eculizumab is effective in decreasing hemolysis and thrombosis, but not marrow failure. Thrombosis 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 pig-A gene that results in hematopoietic cells lacking GPI-linked proteins.
- Patients may experience chronic hemolytic anemia, cytopenias, or a thrombotic tendency.

Key points (continued)

- Flow cytometric techniques to identify cell populations lacking GPI-linked proteins (CD55 and CD59) have replaced the sucrose hemolysis and Ham tests.
- PNH clones have been identified in individuals without hematologic abnormalities.
- Bone marrow failure often precedes or follows clinical PNH.
- Steroid therapy along with supportive measures can ameliorate the hemolytic anemia.
- 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.
- 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.

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 hematocrit of 21% (normal 2 years prior). Corrected reticulocyte count is elevated at 3%, LDH 1,686 IU/dL, and indirect bilirubin 3.4 mg/dL. 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 extravascular as opposed to intravascular hemolysis include the presence of free Hb in the plasma and urine, resulting in red urine and pink plasma. If the hemolysis is chronic, urine

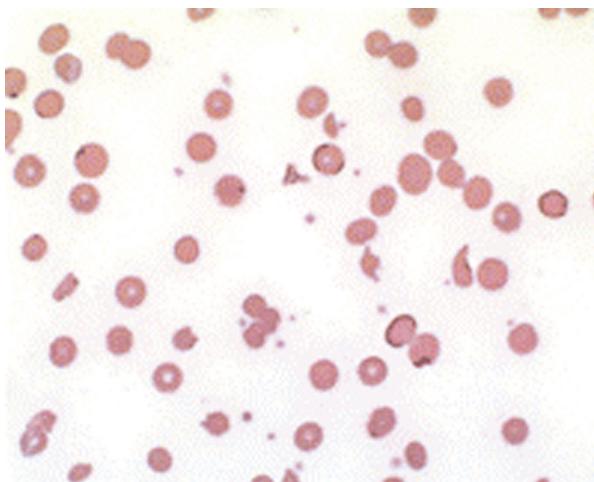


Figure 7-15 Schistocytes.

hemosiderin may be present. In fragmentation hemolysis, schistocytes are a prominent feature of the blood smear (Figure 7-15).

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. When thrombosis is part of the picture, the term thrombotic microangiopathy is used. In disseminated intravascular coagulation (DIC), the microangiopathic hemolytic anemia is accompanied by activation and consumption of soluble clotting factors, resulting in prolongation of the prothrombin time and activated partial thromboplastin time, whereas TTP-HUS is associated with activation of platelets, but not soluble clotting factors. In both processes, thrombocytopenia accompanies the hemolytic anemia, and both thrombosis and bleeding may occur.

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 an unendothelialized 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. Prosthetic valve dysfunction or perivalvular regurgitation may result in intravascular hemolysis, however. 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 bioprosthesis.

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 may include abnormal erythrocytes with schistocytes and cells with abnormal membrane projections.

With chronic hemolysis, Hb 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.

Hemolytic-uremic syndrome–thrombotic thrombocytopenic purpura

HUS and TTP are due to the deposition of platelet microthrombi along the endothelium of small vessels of multiple organs. The classic clinical presentation consists of microangiopathic hemolytic anemia and thrombocytopenia. In advanced stages, fever, renal failure, and waxing mental status changes are seen. TTP may be confused with eclampsia,

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-HUS and DIC is the presence of consumptive coagulopathy in the latter. Malignant hypertension and renal crisis in scleroderma may resemble TTP-HUS, presenting with microangiopathic hemolysis, thrombocytopenia, and renal insufficiency. Rapid control of the hypertension is important in these patients. (TTP and the HUS are covered in detail in Chapter 10.)

Certain drugs, especially antineoplastic agents, can cause microangiopathic hemolysis that resembles TTP and HUS. 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- or HUS-like syndromes.

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. Microangiopathic hemolytic anemia may be present but often is not severe enough to cause morbidity. Disseminated malignancy presents with microangiopathic anemia 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 (microangiopathic hemolysis, elevated liver enzymes, and a low platelet count) is a serious complication of late pregnancy that is part of a spectrum with preeclampsia. Thrombocytopenia and microangiopathic hemolytic anemia with or without renal failure may also occur in pregnancy due to TTP-HUS and AFLP. It is important to distinguish TTP-HUS 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-HUS occurs 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-HUS. Severe liver dysfunction or liver failure favor AFLP.

The characteristics of the coagulopathy are different as well. Whereas both TTP-HUS and HELLP are characterized by thrombocytopenia, in HELLP and more so in AFLP, DIC also may be present with evidence of consumptive coagulopathy. In TTP-HUS, 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 proved helpful in subsequent randomized trials.

Kasabach-Merritt syndrome

Kasabach-Merritt syndrome occurs in young children. It is characterized by consumptive coagulopathy occurring in the capillaries of a large kaposiform hemangioendothelioma. Microangiopathic hemolytic anemia 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

Clinical case (continued)

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 oral iron. Erythropoietin administration also should be considered. 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 workup of liver function test abnormalities. Her recent history has been significant for bizarre schizophrenic-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 Coombs-negative hemolytic anemia. Liver enzymes are moderately elevated. A ceruloplasmin level returns low at 11 mg/dL.

The use of primaquine and dapsone to prevent or treat *Pneumocystis jiroveci* in acquired immunodeficiency syndrome (AIDS) patients 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. Methemoglobinemia and G6PD deficiency are covered in detail earlier in this chapter.

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, 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, Hb, 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, 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 U.S. 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 four 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, respirations 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

Clostridial sepsis 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. Severe neutropenia of any cause may be a significant 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 hemolysis. Brisk intravascular hemolysis with spherocytosis seen on the peripheral blood smear is accompanied by hemoglobinemia, hemoglobinuria, and severe anemia. The plasma may be a brilliant red color, and there may be dissociation between the Hb and hematocrit values because of the

plasma Hb levels reaching several grams per deciliter. Acute renal failure may ensue secondary to excessive hemoglobinuria, but the exact mechanism remains disputed. 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*, *S. 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 *M. pneumoniae* 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 7-14). When ethylenediaminetetraacetic acid–anticoagulated blood is cooled in a test tube, RBC agglutination can be seen; disagglutination occurs when the blood is warmed. Cold agglutination 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 antibody. 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 the formation of a cold-reactive hemolytic IgG antibody of the Donath-Landsteiner type, which induces complement lysis of RBCs.

Microangiopathic hemolytic anemias 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, microvascular thrombi, and fragmentation hemolytic anemia. Patients with either congenital or tertiary syphilis may develop paroxysmal cold hemoglobinuria. Whereas paroxysmal cold hemoglobinuria 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.

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 1–2 weeks later. Hemolysis in malaria results directly from erythrocytic infestation by *Plasmodium* organisms (Figures 7-16 and 7-17). Noninfected RBCs may be hemolyzed by poorly understood mechanisms. Infested erythrocytes are selectively removed from the circulation by the spleen, with some RBCs reentering circulation after splenic pitting of parasites. Previously infested erythrocytes manifest membrane and metabolic abnormalities along with decreased deformability. In addition, the *Plasmodium* species digests the host RBC Hb 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*

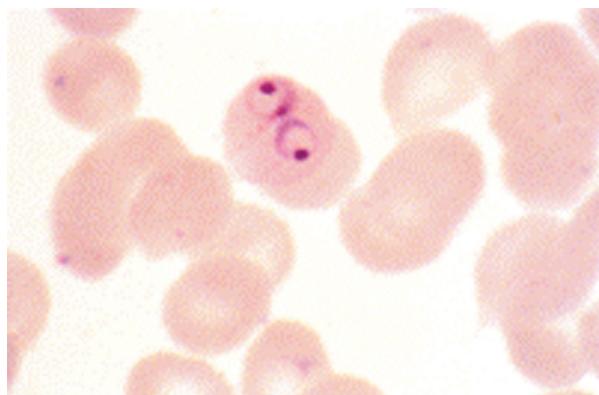


Figure 7-16 Intraerythrocyte parasite *P. falciparum*.

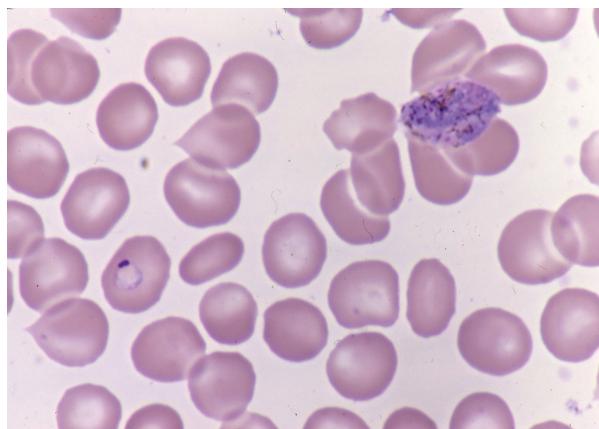


Figure 7-17 Intraerythrocyte parasite *P. vivax*. Trophozoite (ring form) and female gametocyte. Used with permission from *Lichtman's Atlas of Hematology*, <http://www.accessmedicine.com>.

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 polymorphisms 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 *P. falciparum* erythrocyte protein 1 on the membranes of infected RBCs. 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 two 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, 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 1–4 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 7-18). Standard treatment has consisted of clindamycin and quinine. Recent studies have suggested that atovaquone plus azithromycin is an equally efficacious regimen, yet better tolerated.

Bartonellosis

Bartonellosis, caused by *Bartonella bacilliformis*, manifests in two clinical stages: an acute hemolytic anemia and a chronic granulomatous phase. The microorganism enters 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.

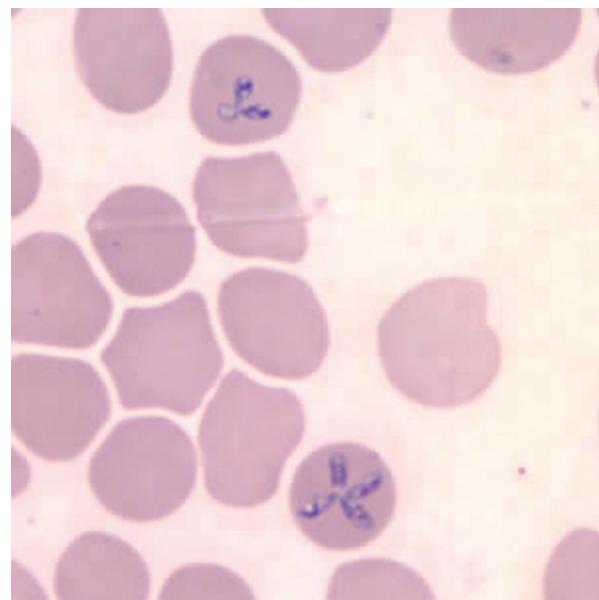


Figure 7-18 Intraerythrocyte parasite *Babesia microti*.

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 *P. 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 U.S. military to screen all personnel for G6PD deficiency.

Key points

- Red cell 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.
- Red cell 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 through alterations in noninfected cells. Malaria can be diagnosed by thorough review of a thick Wright-stained peripheral blood smear.

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Thrombosis and thrombophilia

Stephan Moll, David Y. Garcia and Anjali Alatkar Sharathkumar

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CHAPTER
08



Thrombosis and thrombophilia

Stephan Moll, David Y. Garcia, and Anjali Alatkari Sharathkumar

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Introduction

This chapter gives an overview of the pathophysiologic contributors to thrombosis; describes the mechanisms, epidemiology, testing issues, and clinical relevance of inherited and acquired thrombophilias; discusses the drugs used as anti-thrombotics; and reviews various clinical, diagnostic, and therapeutic aspects of thrombosis.

Pathophysiology of thrombosis

Thrombosis, defined as excessive clotting, has three main causes, referred to as Virchow's triad: reduced blood flow (stasis), blood hypercoagulability, and vascular wall abnormalities. Blood in the vasculature is kept in a fluid state by the delicate balance of multiple procoagulant and anticoagulant factors (ie, coagulation proteins [coagulation factors], platelets, leukocytes, erythrocytes, and components of the vessel wall). If blood vessel integrity is interrupted, coagulation takes place and a blood clot (ie, thrombus) forms to prevent excessive bleeding. Natural anticoagulants, such as antithrombin (AT), protein C, and protein S, serve as "police proteins," controlling and limiting thrombin formation. Once a thrombus has formed, its growth is limited by clot lysis, which eventually leads to thrombus resolution. This is part of the mechanism that leads to

vascular wall healing and reestablishment of intravascular blood flow.

Thrombosis is often multifactorial, caused by both genetic and acquired risk factors. The degree of contribution to thrombosis of the three components of Virchow's triad varies between arterial and venous thrombosis, as well as between thrombotic events in individual patients. Stasis of blood is a well-known predisposing factor for venous thrombosis. The events that trigger an episode of unprovoked (formerly called *idiopathic*) venous thromboembolism (VTE) in a specific location and at a certain time are not well understood. Thrombosis is believed to be multicausal, however, with more than one factor (genetic or environmental) needed for thrombosis to occur.

A pathophysiologic model suggests that each individual has a baseline thrombosis risk, which increases as the person ages [Figure 8-1(a)]. 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 8-1(b)]. Most people, therefore, never reach the thrombosis threshold and never develop a VTE. The individual who has a higher baseline thrombosis risk, such as the individual with an inherited or acquired intrinsic pre-disposition to clotting (thrombophilia), may cross the thrombosis threshold while exposed to a transient risk factor and, thus, will develop a VTE [Figure 8-1(b)].

In general, venous thrombosis is caused by disturbances in the plasma coagulation system with platelet participation playing a 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

Conflict-of-interest disclosure: Dr. Moll: consultancy: GTC Biotherapeutics; ITC; Talecris. Dr. Garcia: consultancy: Bristol-Meyers Squibb; CSL Behring; Daiichi Sankyo. Dr. Sharathkumar declares no competing financial interest.

Off-label drug use: Dr. Moll: not applicable. Dr. Garcia: not applicable. Dr. Sharathkumar: not applicable.

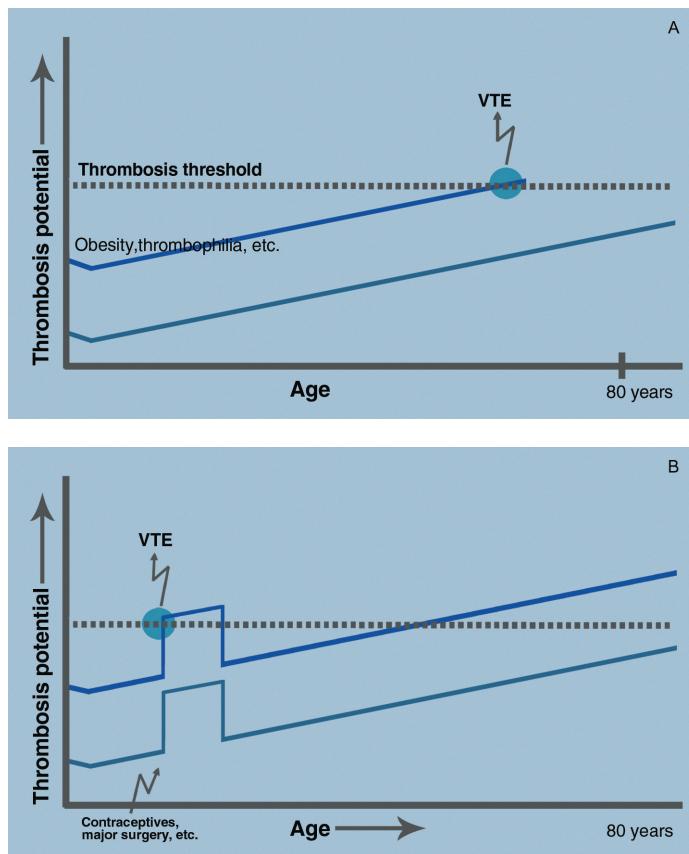


Figure 8-1 Threshold model of thrombosis risk. VTE = venous thromboembolism. Modified after Rosendaal FR. *Lancet*. 1999;353:1167-1173.

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. It also helps explain why antiplatelet drugs are effective in the prevention of arterial thrombosis, but less so in preventing VTE. Thrombus formation in the cardiac ventricles and atria often is caused by stagnant blood flow in dyskinetic, or aneurysmal parts of the heart chambers or in fibrillating atria. Arising in a slow-flow environment, these thrombi more likely are caused by mechanisms similar to the ones that lead to venous thrombosis.

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 arterial ischemic syndromes. Plaques have a high content of tissue factor expressed on monocytes and macrophages. Disruption of the fibrous cap or endothelium overlying an atheromatous plaque leads to exposure of collagen and of tissue factor, leading to platelet adhesion and aggregation and local thrombin formation, with subsequent thrombus formation.

Thrombophilias

The terms *thrombophilia* and *hypercoagulable state* refer to hereditary or acquired predispositions to develop thrombosis.

Factor V Leiden

General information

Activated protein C (APC) is a potent inhibitor of the coagulation system, cleaving the activated forms of factor V and VIII (FVa and FVIIIa) [Figure 8-2(a) and 8-2(b)]. The FVL mutation is a point mutation (G1691A) in the factor V gene, leading to a 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 8-2(c)]. Based on the initial observation that APC did not appropriately prolong the activated partial thromboplastin time (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 antiphospholipid antibodies (APLA), pregnancy, and cancers. FVL is inherited in an autosomal-dominant

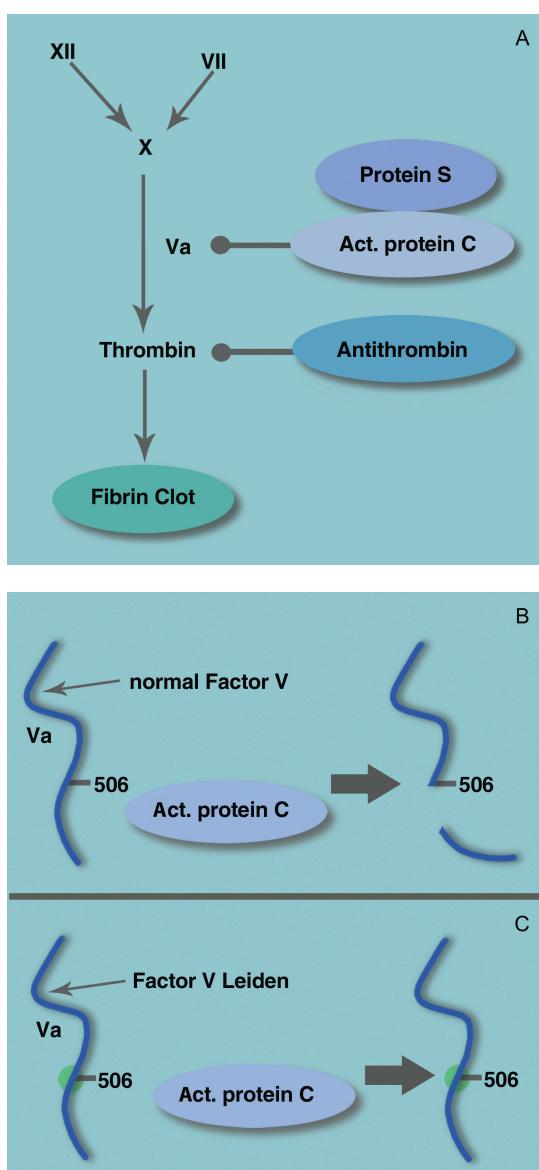


Figure 8-2 A, Sites of action of the natural anticoagulants; B, method of inactivation of factor V; and C, demonstration of the inability of activated protein C, to inactive factor Va when the factor V Leiden mutation is present.

fashion. The high prevalence of FVL in the general population suggests that it has led to evolutionary advantages, perhaps including less blood loss during delivery and improved survival during sepsis.

Prevalence

FVL is the most common known inherited thrombophilia, with a prevalence of 3%-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.

Testing

The diagnosis of FVL is made by genetic testing (ie, polymerase chain reaction [PCR]) testing of the FVL gene. One also can use an APC resistance assay first, however. The currently used second-generation APC resistance assays, which are aPTT-based coagulation assays using factor V-deficient plasma, are sensitive and relatively specific for the 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. A normal APC resistance test result, however, rules out the presence of FVL and does not need to be confirmed by genetic testing. Although clinicians often consider genetic testing to be infallible, false-positive and false-negative results occasionally can occur. These may be due to sample labeling or handling errors, or mistakes in reporting or interpreting the results. A patient's lack of understanding of the test performed and the result obtained, or an error in recall of what the health care provider explained also may lead to misinterpretation and misunderstanding by the patient. Lastly, technical aspects of test performance and rare genetic variations (mutations close to the FVL 506 mutation or the prothrombin 20210 mutation) also may lead to incorrect results.

Risk for thrombosis

Heterozygosity for FVL is mildly thrombophilic, leading to a 2.7-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 use of estrogens, increase the risk further. In a landmark population-based study the lowest and highest absolute 10-year risks for VTE were 1% and 10% in heterozygotes, and 3% and 51% for homozygotes, depending on age, smoking status, and obesity. A systematic review demonstrated that FVL heterozygosity is associated only weakly with an increased risk of VTE recurrence (odds ratio [OR] 1.56; 95% confidence interval 1.14-2.12) compared with individuals without FVL. The risk of recurrence in individuals with homozygous FVL compared with those without FVL is 2.65-fold (95% confidence interval 1.2-6.0) increased. For practical purposes, there is no clinically meaningful association between FVL and arterial thromboembolic events in adults: A meta-analysis demonstrated the risk to be 1.21-fold increased (95% confidence interval 0.99-1.49) in FVL carriers compared with noncarriers.

Management

Because heterozygosity for FVL confers only a mildly increased risk of VTE recurrence compared with individuals

without FVL, its finding alone typically does not alter anticoagulation treatment decisions. As heterozygous FVL is barely a risk factor for arterial thromboembolism, its presence should not influence management decisions. Furthermore, asymptomatic family members of persons with FVL heterozygosity need not be tested unless they are considering estrogen therapy or pregnancy, in which case testing could be considered. Even in this situation, however, there is no strong evidence-based reason for testing, as solid management decisions typically can be made without the knowledge of the mutational status. Finding the homozygote state in a patient with an unprovoked VTE can be one of the arguments to treat with long-term anticoagulation.

Pediatric considerations

As in adults, heterozygosity for FVL is also associated with an increased risk of first VTE. FVL alone, however, does not increase the risk of recurrent VTE. In the homozygous state or if combined with other thrombophilias, the risk for recurrent events is increased. Although FVL is not a clinically relevant risk factor for arterial disease in adults, several pediatric studies have demonstrated an association with stroke, particularly perinatal stroke.

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 common 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%-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 non-Caucasian populations. Homozygosity for the prothrombin 20210 mutations occurs, by calculation, in approximately 1 in 4,000 individuals of Caucasian heritage.

Testing

Testing is done using genetic testing. 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. As discussed for FVL, false-positive and false-negative results with PCR genetic testing can occur.

Risk for thrombosis

Heterozygosity for the prothrombin 20210 mutation is mildly thrombophilic, conferring a threefold increased risk of first-time VTE compared with noncarrier status. A systematic review showed that heterozygosity for the prothrombin 20210 mutation compared with the absence of the mutation is not associated with an increased risk of recurrence of VTE (OR 1.45; 95% confidence interval 0.96-2.2). 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. Meta-analysis has not demonstrated any clinically meaningful association between the prothrombin mutation and arterial thromboembolism. The risk for an arterial event is only 1.32-fold (95% confidence interval 1.03-1.69) increased in carriers of the mutation, compared with noncarriers. There is some suggestion, however, of a somewhat-stronger association between the prothrombin 20210 mutation and stroke and myocardial infarction in younger patients.

Management

Because heterozygosity for the prothrombin 20210 mutation does not confer an increased risk of VTE recurrence, its finding does not alter length of anticoagulation treatment decisions. As the heterozygous prothrombin 20210 mutation is only a very mild risk factor for arterial thromboembolism, its presence should not influence management decisions. Furthermore, similar to the discussion about FVL, family members of people who are heterozygous need not be tested unless they are considering estrogen therapy or pregnancy, in which case testing can be considered.

Pediatric considerations

A recent meta-analysis showed that presence of the prothrombin 20210 mutation leads to an increased risk of recurrent VTE in children, with an odds ratio of 2.15 (95% confidence interval 1.12-4.10). It is unclear at this point if it should alter the duration of therapy.

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 VIIIa, making them unavailable as cofactors during the coagulation process [Figure 8-2(a)]. 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 in 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 pregnancies.

Testing

When evaluating an individual for protein C deficiency, a protein C functional (activity) test should be performed, because obtaining only an antigen levels will miss 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 needs to clarify with the laboratory which test was actually done. Falsey 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 vitamin K antagonists (VKAs) (Tables 8-1 and 8-2). Some authors have advocated the use of a ratio of protein C activity or antigen and levels of other vitamin K dependent factors to help diagnose protein C deficiencies in patients on VKAs. Although such calculations may suggest a deficiency, the poor correlation of coagulation factor levels during VKA treatment makes this an error-prone approach. Patients should be off VKAs for 2-3 weeks before protein C activity testing is performed. 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 in a patient with an “established” 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. Repeat confirmatory testing of a low protein C level at a separate time point is also recommended.

Risk for thrombosis

Protein C deficiency is considered to be one of the higher risk thrombophilias. It is a risk factor mainly for VTE. Rates of thrombosis vary widely among individuals and families with protein C deficiency. A large family cohort study found that the presence of protein C deficiency in asymptomatic relatives of probands with protein C deficiency and a first VTE conferred a 24-fold increased risk compared with family members without the deficiency. The annual incidence of a first VTE is 1.52% in protein C-deficient individuals compared with approximately 0.1% in the general population. Protein C deficiency also is associated with a high risk of recurrent VTE: 37% over 5 years off anticoagulation. A study of thrombophilic families showed that deficiency of protein C is a risk factor for arterial thromboembolism in patients <55 years old. Rates of fetal loss after 28 weeks gestation are increased in individuals with protein C deficiency.

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 after the initiation of VKA. Any patient with acute VTE who is initiated on VKA needs concurrent anticoagulation with a parenteral anticoagulant for at least 5 days and until the international normalized ratio (INR) is >2.0, but this is particularly important in the person with known protein C and S deficiency. Because of the association of protein C deficiency with recurrent thrombosis, consideration of long-term anticoagulation after a first unprovoked thrombotic event is appropriate in these patients. Whether the patient with protein C deficiency and a nonarteriosclerotic arterial thrombotic event would best be treated with antiplatelet or anticoagulant therapy is not known.

Pediatric considerations

Levels of natural anticoagulant proteins change with development. Protein C activity is very low when compared with adults at the time of birth. This reduction of activity can persist until adolescence. When interpreting the results of

Table 8-1 Conditions associated with acquired coagulation factor deficiencies.

Factor	Conditions associated with decreased factor levels
Protein C	<ul style="list-style-type: none"> • Acute thrombosis • Warfarin therapy • Liver disease • Protein-losing enteropathy
Protein S	<ul style="list-style-type: none"> • Acute thrombosis • Warfarin therapy • Liver disease • Inflammatory states • Estrogens (contraceptives, pregnancy, postpartum state, hormone replacement therapy) • Protein-losing enteropathy
Antithrombin	<ul style="list-style-type: none"> • Acute thrombosis • Heparin therapy • Liver disease • Nephrotic syndrome • Protein-losing enteropathy

thrombophilia screening in children, it is imperative that they are compared with pediatric normative ranges and not adult ranges. Homozygous or double heterozygous protein C deficiency is associated with catastrophic thrombotic complications at birth, manifested by purpura fulminans

(extensive microvascular thrombosis of the skin) and, less commonly, massive deep venous thrombosis (DVT). Intrauterine thrombotic events also have been reported in these infants. 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. These newborns need initial treatment with protein C concentrates (plasma-derived protein C concentrates) along with anticoagulants to control and prevent the progression of thrombosis. Neonates and children with severe inherited protein C deficiency have an ongoing risk of purpura fulminans and, therefore, require long-term antithrombotic therapy. The risk of thrombosis is very low in children with heterozygous protein C deficiency. Counseling these families about avoiding exposure to transient risk factors of VTE and its signs and symptoms is critical. Adolescent girls with known protein C deficiency should be discouraged from using hormonal therapy for birth control and should be offered birth control options without thrombotic risk.

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%

Table 8-2 Influence of acute thrombosis, heparin, and vitamin K antagonists on thrombophilia test results.

Test	Acute thrombosis	Unfractionated heparin	Low molecular weight heparin	Vitamin K antagonists
Factor V Leiden genetic test	reliable	reliable	reliable	reliable
APC ¹ resistance assay	reliable ²	???	???	reliable ²
Prothrombin 2010 genetic test	reliable	reliable	reliable	reliable
Protein C activity or antigen	???	reliable	reliable	low
Protein S activity or antigen	may be low	reliable	reliable	low
Antithrombin activity	may be low	may be low	may be low	occasionally elevated ⁷
Lupus anticoagulant	reliable ⁵	???	???	may be false positive
Anticardiolipin antibodies	reliable ⁵	reliable	reliable	reliable
Anti-β ₂ -glycoprotein I antibodies	reliable ⁵	reliable	reliable	reliable
Homocysteine	reliable	reliable	reliable	reliable

¹APC = activated protein C.

²Reliable if the assay is performed with factor V depleted plasma; thus: clinician needs to inquire how the individual laboratory performs the assay.

³Depending 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 on heparin.

⁴Probably reliable, but limited data in literature.

⁵Test often positive or elevated at time of acute thrombosis, but subsequently negative.

⁶While many test kits used for lupus anticoagulant testing contain a heparin neutralizer making these tests reliable on unfractionated heparin (UF) and possibly low molecular weight heparin (LMWH), clinicians needs to inquire with their laboratory how their individual test kit performs in samples with UF and LMWH.

⁷A few case reports show that VKA can lead to an increase in AT levels in selected families.

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 for the inactivation of factors Va and VIIa [Figure 8-2(a)].

Protein S deficiency was first described in 1984. More than 131 different mutations have been identified leading to protein S deficiency. Protein S deficiency 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.

Prevalence

Reported prevalences in the general population vary between 1 in 800 and 1 in 3,000, but due to difficulties in establishing the normal range of protein S concentrations in the general population and the difficulties in making an accurate diagnosis, the true prevalence of protein S deficiency is not known.

Testing

A reliable test for quantitative protein S deficiency is the determination of free protein S antigen levels. If only antigen levels are determined, however, a type II protein S deficiency can be missed. If only activity is determined, some patients with quantitative and also qualitative protein S deficiency may not be discovered, because some activity assays have been shown to give falsely normal results. Therefore, it is advisable to include functional testing (protein S activity) and immunologic testing (free protein S antigen) in the laboratory evaluation of suspected protein S deficiency. Obtaining a total protein S antigen is helpful only to determine the subtype of protein S deficiency, but for clinical purposes such classification is not needed or helpful. 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, liver disease, nephrotic syndrome, disseminated intravascular coagulation (DIC), and therapy with VKAs (Tables 8-1 and 8-2). Congenital protein S deficiency cannot be diagnosed in these circumstances. A patient needs to have been off VKAs for 3 weeks before protein S levels can be considered reliable. Thus, as with protein C deficiency, timing of the testing is essential to make a correct diagnosis, and repeat confirmatory testing on a new plasma sample is advisable. Critical questioning as to whether a patient said to have protein S deficiency truly has the disorder is appropriate.

Risk for thrombosis

Protein S deficiency is 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. A large family cohort study found that presence of protein S deficiency in asymptomatic relatives of probands with protein S deficiency, and a first VTE conferred a 31-fold increased risk compared with family members without the deficiency. The annual incidence of first VTE is 1.9% in protein S-deficient individuals, compared with approximately 0.1% in the general population. Interestingly, some authors have not found an increased risk of thrombosis in individuals with inherited protein S deficiency. Nevertheless, protein S deficiency is considered to be a higher risk thrombophilia because the risk of recurrent VTE off anticoagulation has been shown to be high, reaching 44% over 5 years. A large family study showed that protein S deficiency is a risk factor for arterial thromboembolism in individuals <55 years of age. Clearly, there is heterogeneity in the clinical phenotype of patients with protein S deficiency, and this needs to 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 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. Individuals with a first unprovoked episode of VTE are often treated long term with VKAs because of an increased risk for recurrent VTE if the patient is not on VKAs. Whether the patient with protein S deficiency and an otherwise-unexplained arterial thromboembolic event should best be treated with antiplatelet or anticoagulant therapy is not known. No protein S concentrate exists.

Pediatric considerations

Purpura fulminans can occur in the rare newborn with severe protein S deficiency because of homozygous or double heterozygous mutations. Children are born with physiologically lower levels of total protein S than adults. Because the amount of C4b BP also is reduced, however, the free protein S level is almost the same as found in adults. Any reduction of protein S level or activity in healthy newborns should normalize by early childhood (after 6 months of age). Screening asymptomatic children for thrombophilia should be delayed until after this time so that testing does not need to be repeated. Clinical presentation of homozygous or double-heterozygous protein S deficiency is similar to severe protein C deficiency. 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.

Antithrombin deficiency

General Information

AT is an enzyme that interrupts the coagulation process mostly by inhibiting thrombin [Figure 8-2(a)], 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. Qualitative (type I) and quantitative (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, thus termed pleiotropic defects. 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. Acquired AT deficiency is associated with sepsis, DIC, liver disease, the nephrotic syndrome, asparaginase chemotherapy, and acute fatty liver of pregnancy.

Testing

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 8-2). Warfarin can increase AT levels, possibly by stimulating its synthesis or reducing its consumption by decreasing low-grade activation of the coagulation process. This increase can be minimal and for clinical decision making is not relevant; however, substantial AT increase on VKA also has been reported, but it is not known how often that occurs. Testing is best performed a few weeks after the initial thrombotic event and may best be done when a patient is not on anticoagulants. No one should be diagnosed as having AT deficiency on the basis of one single abnormal test result. 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. The prevalence of VTE in individuals with a defect in the heparin-binding site is much lower; only 6% of such individuals will develop a VTE. Once anticoagulation is stopped, the risk of recurrent VTE in individuals with AT deficiency is high, between 10% and 17% per year. Although some cases of arterial thromboembolism in AT-deficient individuals have been reported, this association is much weaker than with VTE and possibly is not present at all. A large family study showed no association between AT deficiency and arterial thromboembolism. The risk for fetal loss is slightly increased in women with AT deficiency.

Management

Asymptomatic individuals with AT deficiency typically are not started on long-term anticoagulation. They need VTE prophylaxis in high-risk situations, however. AT concentrate is available, either derived from the plasma of human donors or transgenically produced in goat milk. Only one guideline or consensus statement exists as to when to use AT concentrates. In view of the scarcity of solid clinical study data, this guideline includes only level IIIC recommendations (ie, “opinions of respected authorities, based on clinical experience, descriptive studies”). The guideline provides detailed recommendations on how to dose with plasma-derived AT concentrate, but it lacks specifics on whom to treat and for how long. Because the risk of recurrent VTE is high, it typically is recommended that a patient with AT deficiency who

has had an unprovoked VTE should be considered for long-term anticoagulation. It is not known whether the same recommendation should apply to patients with AT deficiency resulting from a defect in the heparin-binding site. AT deficiency occasionally confers resistance to anticoagulation with heparin. Large doses of unfractionated heparin (UFH) can be required to achieve appropriate prolongation of the aPTT. In cases of severe thrombosis and inadequate anticoagulation, AT concentrate can be given.

Pediatric considerations

AT levels are reduced at birth and normalize by approximately 6 months of age. Screening of asymptomatic children should be delayed until this time to avoid needing repeat testing. Although risk of thrombosis is very low in children with confirmed deficiency of AT, it is critical that these families should be counseled about avoiding exposure to transient risk factors of VTE and its signs and symptoms.

Antiphospholipid antibodies

General information

APLAs are acquired autoantibodies targeted against phospholipids and phospholipid-binding proteins, such as β_2 -glycoprotein-I and prothrombin. They are associated with arterial thromboembolism and VTE as well as pregnancy loss. A variety of different mechanisms leading to thrombosis have been proposed, including effects of the antibodies on platelets, endothelial cells, monocytes and trophoblasts, and interference with complement activation, the protein C pathway, and fibrinolysis. Clinical classification of the APLA syndrome requires a history of venous or arterial thrombosis, unexplained recurrent early pregnancy loss, or one or more late pregnancy losses, together with persistent laboratory evidence of APLA, tested at least 12 weeks apart. The criteria for definite APLA syndrome have been described as the so-called Sapporo criteria. The syndrome occurs as primary APLA syndrome not associated with any other diseases, or as secondary APLA syndrome associated with autoimmune diseases, malignancy, or drugs.

Prevalence

The prevalence of APLA syndrome is poorly defined, but APLAs are found in nearly 50% of patients with systemic lupus erythematosus (SLE) and 1%-5% of the general population. Nearly 40% of patients with SLE will meet diagnostic criteria for the APLA syndrome.

Testing

The revised Sapporo criteria recognize the following antibodies as fulfilling criteria for APLA syndrome: (i) moderately or

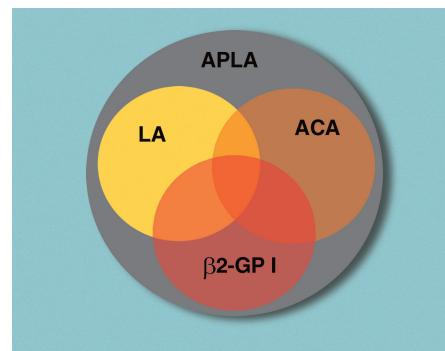


Figure 8-3 Antiphospholipid antibodies (APLAs) with their different subtypes. ACA = anticardiolipin antibody; β_2 -GPI = anti- β_2 -glycoprotein I antibodies; LA = lupus anticoagulant.

highly positive immunoglobulin G (IgG) and immunoglobulin M (IgM) anti- β_2 -glycoprotein I antibodies; (ii) moderately or highly positive IgG and IgM anticardiolipin antibodies; and (iii) lupus anticoagulant (Figure 8-3). Lupus anticoagulants are detected by various functional coagulation assays, because the APLAs react with the phospholipids needed for the ex vivo coagulation process, and, thus, prolong clotting times. False-positive lupus anticoagulant test results are not uncommon, occurring more frequently in patients who (i) are on oral anticoagulants, (ii) are older, and (iii) have mildly positive lupus anticoagulant test results (Table 8-2). False-negative results may occur if the blood sample was suboptimally centrifuged and the 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, the revised Sapporo criteria require repeatedly positive tests at least 12 weeks apart to confirm a diagnosis of APLA syndrome.

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 anti- β_2 -glycoprotein I antibodies, antiphosphatidylserine antibodies, antiphosphatidylethanolamine antibodies, and antiphosphatidylinositol antibodies. There is presently no clear indication for testing for these additional APLA in routine clinical practice. In fact, evidence to support anticardiolipin IgG and IgM antibody elevation alone as risk factors for thrombosis is lacking. Nevertheless, they are part of the empirically derived revised Sapporo criteria. The different anticardiolipin and anti- β_2 -glycoprotein I antibody test kits available for clinical practice are suboptimally standardized. Also, lupus anticoagulant reporting is not standardized, and laboratory reports can be difficult to read and interpret. Thus, critical and diligent

reading of the report is advised when interpreting a laboratory lupus anticoagulant report.

The INR determined from plasma occasionally is invalid in patients on VKAs because of a lupus anticoagulant effect on the INR. Furthermore, for patients with APLAs, INR determinations by point-of-care INR monitors often are inaccurate and significantly overestimate a patient's level of anticoagulation. Alternative tests, such as chromogenic factor X or clot-based factor II or X activity to measure the VKA effect are indicated in these patients. The target ranges for these tests depend on the reagents and instruments used for their determination, but an INR range of 2.0-3.0 in a non-APLA patient on VKA corresponds to a factor II activity of approximately 31%-15% and chromogenic factor X activity of approximately 42%-21%.

Risk for thrombosis

The APLA syndrome is highly thrombophilic and is associated with both arterial and venous thrombosis. Positivity for all three APLA tests (ie, lupus anticoagulant, anticardiolipin, and anti- β_2 -glycoprotein I antibody tests) is associated with the highest risk for thrombosis and pregnancy loss. APLA syndrome also is implicated in recurrent VTE once anticoagulation is discontinued. Limited data exist on the extent to which APLAs increase the risk of recurrence, particularly in individuals' whose index event was provoked. It also is not established which APLA types best predict recurrent thrombosis. Finally, there is a 5%-15% failure rate of warfarin therapy in preventing recurrent thrombosis.

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 APLA syndrome 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 true APLA syndrome 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 UFH 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 either a factor II activity or 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 APLA syndrome, results of the POC instrument should be correlated with phlebotomy 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 4-6 months. It is not known, however, what the optimal frequency of such recorrelation is.

It is not known whether patients with arterial thrombosis and APLA syndrome are more effectively treated with anti-platelet or VKA anticoagulation therapy. In the absence of prospective randomized trial data, no consensus on this topic exists. 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 has not been studied. The management of pregnant women with APLA is discussed elsewhere in this self-assessment program.

Pediatric considerations

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 of 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 (~3-6 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 APLA syndrome 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.

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.

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 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.

Testing

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 elevations in factor VIII >150% confer a 4.8 times greater risk for first-episode VTE than if levels are <100%. 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

As the role of elevated factor VIII levels in recurrent VTE is controversial, decision on duration and intensity of

anticoagulation should be made independent of factor VIII levels. Consequently, at present, there is no role for routine clinical testing for factor VIII levels as part of a thrombophilia workup.

Pediatric considerations

There is suggestion that persistently elevated FVIII activity, particularly when associated with persistently elevated D-dimers, has prognostic significance in children. These children have higher rates of postthrombotic syndrome and recurrent thrombotic events. It may be that it is beneficial to treat these patients with extended anticoagulation, but this has not yet been investigated.

Homocysteine and MTHFR

General information

Homocystinuria is a rare autosomal recessive defect in the homocysteine pathway (Figure 8-4), most commonly in the cystathione- β -synthase enzyme and is associated with markedly elevated homocysteine levels (>100 μ M/L). The worldwide prevalence of cystathione- β -enzyme deficiency based on newborn screening 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. Mild to moderately elevated homocysteine levels, on the other hand, are common, and are referred to as hyperhomocysteinemia. Elevated levels may be

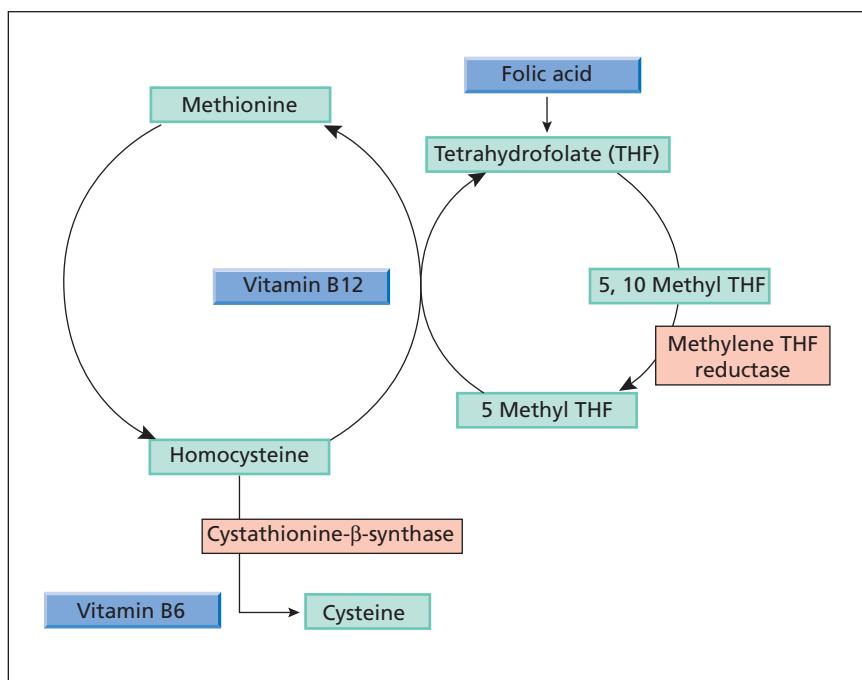


Figure 8-4 Homocysteine pathway.

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. In hyperhomocysteinemia, the associated signs and symptoms seen in homocystinuria do not occur.

Elevated levels of plasma homocysteine have been shown to be associated with an increased risk of venous and arterial thrombosis. From available data it is not clear whether this association is independent of confounding effects or causal in nature. A number of prospective studies have demonstrated that lowering the homocysteine levels does not decrease the risk of primary venous and arterial thromboembolic events or of recurrent venous and arterial thrombosis. This is also true for patients with elevated homocysteine levels secondary to chronic renal disease. This implies that hyperhomocysteinemia may not be causatively contributing to the thrombotic process, but rather act as a marker for an increased risk. The methylenetetrahydrofolate reductase (MTHFR) enzyme is a regulator of homocysteine metabolism (Figure 8-4). 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 “thermolaabile” mutation, for which approximately 34%-37% of U.S. whites are heterozygous and 12% are homozygous. The A1298C polymorphism occurs in most ethnic groups and is present in the heterozygous state in 9%-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.

Testing issues

Homocysteine levels may change after food intake, but the change typically is <10% from baseline, which, for practical purposes outside of clinical studies, is not relevant.

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 screen 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.

Myeloproliferative disorders

General information

Essential thrombocythemia (ET) and polycythemia vera (P 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 P vera and in 50% of those with ET. Meta-analyses 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. At present, however, there are no data to suggest that therapeutic anticoagulation decisions should be based on the presence or absence of the mutation.

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 JAK2 V617F mutation-positive patients have an overt myeloproliferative disorder (MPD) at the time of the diagnosis of their thrombotic event. JAK2 V617F mutation-positive patients with splanchnic vein thrombosis are more likely to develop an MPD 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 MPD. One can similarly argue that the JAK2 V617F mutation-negative patients should be followed just as closely, because up to 10% of these patients also will develop an MPD.

Other VTEs and JAK2 V617F mutation

In patients with nonsplanchnic vein thrombosis, the prevalence of the JAK2 V617F mutation is <1%. The presence of the JAK2 V617F mutation without symptoms of an MPD is not associated with increased risk of recurrent VTE or

progression to an MPD over a 4-year follow-up period. This argues against screening patients with nonsplanchic vein thrombosis for the JAK2 V617F mutation.

Paroxysmal nocturnal hemoglobinuria

General information

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal bone marrow disorder resulting from an acquired mutation of the phosphatidylinositolglycan class A gene in a hematopoietic stem cell, 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 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. A number of etiologies have been hypothesized, including: (i) episodic hemolysis leading to an increase in circulating procoagulant microparticles derived from complement-injured CD55- and CD59-deficient monocytes and macrophages, or from platelets and endothelial cells; (ii) release of free hemoglobin during hemolysis, which activates the endothelium and scavenges nitric oxide; (iii) complement-mediated platelet activation; and (iv) decreased fibrinolytic activity. For prevention and treatment of PNH-associated thrombosis anticoagulation is the treatment option. In addition, long-term treatment with the complement inhibitor eculizumab (Soliris®), approved by the Food and Drug Administration (FDA) in March 2007, has been demonstrated to reduce the risk of clinical thromboembolism in patients with PNH.

Management

Screening for PNH by peripheral blood flow cytometry for CD55 and CD59 is warranted in thrombophilia evaluations

of patients with venous thrombosis and unexplained hemolysis or peripheral blood cytopenias. The various options in preventing and treating thrombosis in this rare disorder have been discussed in detail.

Abnormalities in fibrinolysis

A variety of parameters of fibrinolysis (Figure 8-5) 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 indecisive 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, work-up for abnormalities in the fibrinolytic pathway (ie, testing for plasminogen, tissue plasminogen activator [tPA], plasminogen activator inhibitor-1 [PAI-1], and thrombin-activatable fibrinolysis inhibitor [TAFI]), with the knowledge we have at present, is not meaningful. 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.

Plasminogen

Although plasminogen deficiency initially was believed to be a risk factor for thrombosis, more recent and cumulative data indicate that it does not lead to an increased risk for arterial or venous thrombosis. Thus, at present, plasminogen deficiency should not be considered a hypercoagulable state. Accordingly, there is no role for including plasminogen antigen or activity determination in a thrombophilia work-up. Homozygous or double heterozygous defects in the plasminogen gene are associated with severe plasminogen deficiency and ligneous deposits in various tissues, such as the conjunctiva.

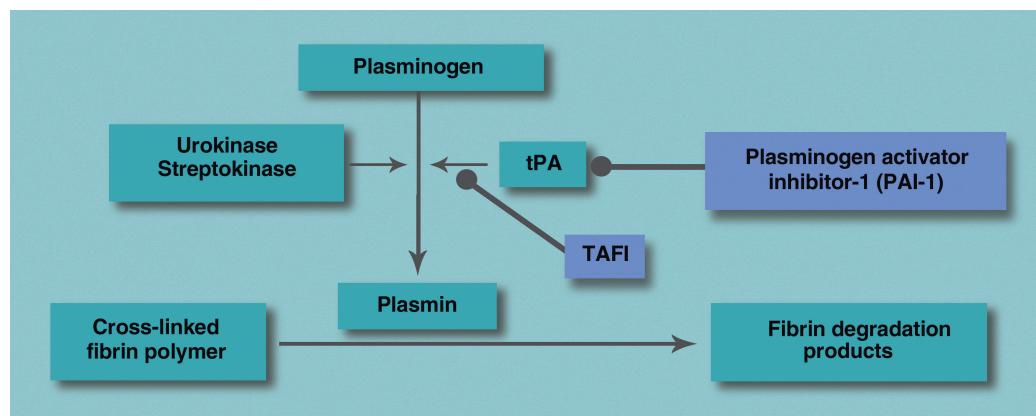


Figure 8-5 Fibrinolysis.

TAFI = thrombin activatable fibrinolysis inhibitor; tPA = tissue plasminogen activator.

Tissue plasminogen activator

Increased tPA antigen levels have been found to increase the risk for arterial thrombosis in some, but not all, studies. No relationship with venous thrombosis has been detected. The observation that elevated tPA levels are associated with arterial thrombosis appears paradoxical. It has been speculated, however, that this association may reflect an association of high PAI-1 levels and arterial thrombosis, which is the principal inhibitor of tPA. Surprisingly, though, the association between PAI-1 levels and arterial thrombosis has been conflicting and unconvincing. Several polymorphisms in the tPA gene have been described, but no clear association between these changes and arterial thrombosis or VTE has been found.

Plasminogen activator inhibitor-1

PAI-1 is the principal inhibitor of tPA. Although some inconsistent study findings exist regarding the association of elevated levels of PAI-1 and the risk of VTE, overall it appears that increased levels are not a risk factor for VTE. The 4G/5G I/D polymorphism of the PAI-1 gene promoter is the most frequently studied polymorphism of PAI-1 and has been shown to be associated with elevated PAI-1 antigen levels. Data on the association of the polymorphism with VTE have been inconsistent. Results of studies on the relationship between PAI-1 and arterial thrombosis also are conflicting and unconvincing. At this point, there is no clinical utility in measuring PAI-1 activity or antigen levels or looking for PAI-1 polymorphisms when performing a thrombophilia work-up in routine clinical practice.

Thrombin-activatable fibrinolysis inhibitor

TAFI suppresses fibrinolysis by cleaving residues from fibrin, thus interfering with the binding of plasmin to fibrin. Although a number of studies have shown an association between elevated TAFI levels and first or recurrent VTE and with arterial thrombosis, not all studies have shown consistent results. There is no role for routine clinical testing for TAFI levels.

Others

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. Although individual studies in adults on the association of Lp(a) and VTE have not shown consistent findings, a recent meta-analysis demonstrated that elevated levels are a mild risk factor for VTE. Similarly, studies of children show an association of elevations in Lp(a) with VTE.

Family history of VTE

Simply having a family history of VTE is a risk factor for first-time VTE, no matter whether a thrombophilia is detectable in the family. This additional risk is due to unknown or unmeasured risk factors. Having one first-degree relative with a history of VTE increases an individual's risk of VTE 2.2-fold; having ≥ 1 affected relatives 3.9-fold. Young age of the affected relative and the number of affected relatives more strongly indicate a predisposition to develop 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.

Others

Increased plasma coagulation factor IX and XI and fibrinogen have been shown to be risk factors for a first episode of VTE, but whether they influence the risk of recurrent VTE is not clear. Similarly, non-O blood group is a VTE risk factor. Dysfibrinogenemias are rare disorders, leading to either a thrombotic or a bleeding tendency. Elevated fibrinogen is a risk factor for arterial thromboembolism. Platelet glycoprotein polymorphisms have not been shown consistently to be risk factors for arterial thromboembolism.

Acquired conditions

A number of environmental and medical conditions lead to an increased risk for VTE.

Cancer

Approximately 20% of all VTEs occur in patients with cancer. The risk for VTE in cancer is determined by a number of coexisting factors, which can be divided broadly into general risk factors (eg, age, obesity, past history of VTE, family history of VTE, coexisting medical conditions, inherited and acquired thrombophilias) and cancer-specific risk factors (cancer type, stage, type of chemotherapy, hormonal therapy, surgery, central venous catheters). About 6% of patients with unprovoked VTE have a previously undiagnosed cancer at the time of the VTE, and about 10% of patients with

unprovoked VTE will be diagnosed with a cancer in the year following the cancer diagnosis. It is not known whether extensive screening for cancer of the patient with unprovoked VTE is beneficial and leads to decreased cancer-associated morbidity or to improved survival. 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, but routine extensive screening for underlying cancer in all patients with unprovoked VTE is not recommended. 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 therapy.

Hormonal therapy, pregnancy

The increased VTE risk associated with hormonal contraceptive, pregnancy, and hormone replacement therapy is discussed elsewhere in this ASH-SAP.

Others

Thrombosis may occur as a complication of systemic or local infection. Head and neck infections may trigger cerebral and sinus vein thrombosis. Liver disease will lead not only 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 anticoagulants (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 because of the need for cardiac catheterizations, hospitalization, and major surgeries.

Whom to test

Consensus guidelines

No general consensus exists as to which patients and family members should be tested for thrombophilias. Clinician-experts continue to disagree about the role of thrombophilia testing in the care of patients with thrombosis that is otherwise unexplained. Several guidelines exist, produced by societies or expert panels, and they vary in their recommendations as to who should be tested and who should not.

Reasons to test or not test

Thrombophilia testing often is considered for patients who (i) experience unprovoked thrombosis at a young age (<50

years), (ii) experience unprovoked thrombosis at an unusual site, (iii) have a history of VTE in one 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 (gender, post-treatment D-dimer, family history). A variety of reasons for and against testing can be quoted (Table 8-3). In the United States, health insurance and employment discrimination based on a person's genetic testing results is illegal, as signed into law in May 2008 (Genetic Information Nondiscrimination Act [GINA]). Life insurance implications—denial of insurance or higher premiums to be paid—based on genetic results is not included into GINA, however, and therefore may occur. Thus, critical consideration should be given to which individual to test for the genetic thrombophilias. In the 2012 American College of Chest Physicians (ACCP) guidelines on *Antithrombotic Therapy for VTE Disease* the absence or presence of thrombophilia did not influence recommendations on duration of anticoagulant therapy in patients with VTE, as thrombophilias as a group were assessed to not be strong or consistent enough risk factors to meaningfully predict recurrence of VTE.

Negative thrombophilia testing in an individual with a family history of thrombophilia does not guarantee protection

Table 8-3 Reasons for and against thrombophilia testing.

A. Reasons for testing

- *Patient with thrombosis*
 - Influence on length of anticoagulation therapy?
 - Partial explanation (for patient and physician) why thrombosis occurred
- *Asymptomatic individual (family member)*
 - Start of long-term primary prophylaxis?
 - Different venous thromboembolism prophylaxis in risk situations?
 - Different choice regarding birth control or hormonal therapy?
 - Different management during pregnancy?
 - Encouragement for lifestyle changes (weight loss, smoking cessation, increased physical activity)

B. 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
- Life insurance implications
- 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?

Table 8-4 Author's (SM) approach which family members of a patient (proband) to consider for thrombophilia testing.

	Male	Female		
	Proband's sons	Proband's brothers	Proband's daughters	Proband's sisters
Proband's thrombophilia				
Hetero FVL or hetero II20210	No testing	No testing	No testing	No testing
Homo FVL or homo II20210	No testing	Reasonable to test	No testing	Test
Double hetero or compound thrombophilia	Reasonable to test	Reasonable to test	Test	Test
Protein C, S, or AT deficiency	Reasonable to test	Reasonable to test	Test	Test

For a detailed discussion, see the text. "Reasonable to test": consider VTE prophylaxis with airline travel, cast, nonmajor surgery; prolonged prophylaxis after major surgeries. "Test": advise against estrogen therapy; give ante- and postpartum anticoagulation, if strong thrombophilia found.

from thrombotic events, as it has been shown that family history of VTE by itself is a risk factor for VTE.

Authors' approach

In view of the absence of generally accepted testing guidelines, the approach presented here is that of the authors of this chapter (approaches are denoted with authors' initials).

An adult hematologist's approach (S. M. and D. G.)

The main reason why we test patients is to detect a higher risk thrombophilia (APLA syndrome, AT deficiency, homozygous FVL, double heterozygous FVL plus prothrombin 20210 mutation, protein C deficiency, and protein S deficiency, and perhaps homozygous prothrombin 20210 mutation) (Table 8-6). Although there is no agreed-on definition of higher risk thrombophilia, one could define it as one with an annual VTE incidence of >5% or a cumulative 5-year VTE recurrence rate, if off anticoagulation, of >25%-30%. The finding of a higher risk thrombophilia has a number of consequences in our practice: (i) it decreases our threshold to recommend long-term anticoagulation in a patient who has had an episode of unprovoked VTE; (ii) it leads to discussion with the patient with an unexplained arterial, nonarteriosclerotic thromboembolic event, whether anticoagulant or antiplatelet therapy might be the preferred treatment for secondary prevention; and (iii) it prompts consideration of testing for the identified thrombophilia(s) in asymptomatic female family members (Table 8-4) and advice against the use of estrogen birth control methods and for anticoagulation prophylaxis during the postpartum, and possibly the antepartum period. When we order thrombophilia testing, we do not test for parameters of fibrinolysis, individual procoagulant factor levels (factors VIII, IX and XI or fibrinogen) or the MTHFR polymorphisms. We limit homocysteine testing to the individual <30 years of age with thrombosis to assess for the presence of homocystinuria. Table 8-5 lists the thrombophilia tests that we order when evaluating a patient for thrombophilia. In general, thrombophilia testing should

be carried out by expert clinicians after a thorough discussion with the patient regarding the possible benefits and limitations of such testing.

A pediatric hematologist's approach (A. A. S.)

In asymptomatic healthy children or girls considering hormonal contraception, thrombophilia testing is not recommended. For children with documented thromboembolic disease, thrombophilia testing is controversial and no general consensus exists when and what to test. I do not perform thrombophilia testing in children who develop VTE due to exposure to transient VTE risk factors. Although a recent meta-analysis in children with inherited thrombophilia demonstrated that inherited thrombophilia, with the exceptions of FVL and Lp(a) elevation, increases a child's risk for recurrent VTE, there is no evidence that screening and identifying the inherited thrombophilia rather than practicing standard secondary VTE prophylaxis in any person who has had one VTE event will reduce the rate of recurrence or

Table 8-5 Thrombophilia tests to consider if decision on thrombophilia workup is made.

A. Venous thromboembolism

- CBC
- Factor V Leiden
- Prothrombin 20210 mutation
- Protein C activity
- Protein S activity, free protein S antigen
- Antithrombin activity
- Anticardiolipin IgG and IgM antibodies
- Anti-β₂-glycoprotein-I IgG and IgM antibodies
- Lupus anticoagulant
- JAK2 V617F and PNH in splanchnic vein thrombosis
- Lipoprotein(a) (in pediatrics)

B. Arterial thromboembolism, unexplained

- See Table 8-11

CBC = complete blood count; IgG = immunoglobulin G; IgM = immunoglobulin M; JAK2 = Janus kinase-2; PNH = paroxysmal nocturnal hemoglobinuria.

Table 8-6 When to consider thrombophilia testing.

- Selected unprovoked VTE, when finding a strong thrombophilia helps with the decision on how long to treat with anticoagulation
- Thrombosis at an unusual site (splanchnic, sinus/cerebral, or renal veins)
- Strong family history of VTE
 - Asymptomatic individual (see Table 8-4) with family history of strong thrombophilia:
 - Antithrombin deficiency
 - Protein C deficiency
 - Protein S deficiency
 - Homozygous factor V Leiden
 - Homozygous prothrombin mutation
 - Compound thrombophilias
- Recurrent VTE while adequately anticoagulated (looking for APLA)
- Unexplained arterial thromboembolism in a young person (ie, no arteriosclerosis risk factors, no cardioembolic source) (see Table 8-11)
- ≥3 unexplained pregnancy losses before week 10, or ≥1 loss after week 10

APLA = antiphospholipid antibodies; VTE = venous thromboembolism.

postthrombotic syndrome. I consider thrombophilia testing in children who present with unprovoked DVT, thrombosis at unusual site, extension of thrombosis despite adequate therapy, and strong suspicion of a higher risk thrombophilia, specifically APLA syndrome and homozygous protein C and S deficiency and AT deficiency, as this information can influence treatment decisions and VTE outcome: (i) a patient with AT deficiency may need AT concentrates to achieve therapeutic anticoagulation with heparin derivatives; and (ii) a patient with severe protein C deficiency will need replacement with protein C concentrates for the treatment of thrombosis. Although infants who present with stroke in newborn period have a higher incidence of thrombophilic traits, the risk of thrombosis recurrence is negligible. I do not perform thrombophilia testing in this population.

Interpreting test results, 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 8-1 and 8-2). 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>; <http://www.stoptheclot.org>).

Antithrombotic drugs

Venous thrombosis occurs mostly via activity of the plasma coagulation system, with only minor platelet participation. In contrast, platelets play a major role in arterial thrombus formation, with the plasma coagulation system participating some. This paradigm helps explain why drugs that block the plasmatic coagulation reaction (ie, anticoagulants) are active in prevention of VTE and also effective in preventing arterial thrombosis, whereas antiplatelet drugs, which successfully prevent arterial thrombosis, are less or not at all effective in venous disease. Thrombus formation involves three steps: (i) platelet adhesion, (ii) platelet aggregation, and (iii) plasmatic coagulation. The natural anticoagulant system (AT, protein C and protein S, tissue factor pathway inhibitor) prevents excessive thrombus formation. The fibrinolytic system (tPA, plasminogen) degrades fibrin and prevents excessive clot formation, and facilitates clot breakdown. The group of antithrombotic drugs consists of anticoagulants, antiplatelet agents, and fibrinolytics.

Antiplatelet agents

Aspirin

Aspirin (acetyl-salicylic-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 nonaspirinated platelets can activate aspirinated platelets. Complete inactivation of platelet COX-1 typically is achieved with a daily dose of aspirin of 160 mg. When used as an antithrombotic drug, aspirin is maximally effective at doses between 50 and 325 mg per day. Higher doses do not improve efficacy. There is, however, considerable interindividual variability in aspirin's ability to inhibit COX-1.

Phosphodiesterase inhibitors

Dipyridamole (Persantine)

Dipyridamole (Persantine) 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 reversible.

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 (Pletal)

Cilostazol (Pletal) 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 (Trental)

Pentoxyphylline (Trental) 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 (Plavix) and ticlopidine (Ticlid)

Clopidogrel (Plavix) and ticlopidine (Ticlid) 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 8–11 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 equally 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 metabolizer to a different antiplatelet agent is clinically beneficial.

Prasugrel (Effient), ticagrelor (Brilinta), and cangrelor

Prasugrel (Effient), ticagrelor (Brilinta), 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 lead to more complete inhibition of platelet function. Prasugrel irreversibly inhibits the platelet P2Y₁₂ receptor, Ticagrelor reversibly. Prasugrel was FDA approved in 2009, Ticagrelor in 2011. Cangrelor was not FDA approved as of January 2013.

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 (ReoPro)

Abciximab (ReoPro) 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 18–24 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 (EDTA)-containing blood tubes can be seen in patients treated with the drug, leading to pseudothrombocytopenia when platelets are counted 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 (Integrilin)

Eptifibatide (Integrilin) 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, thus, 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 6–12 hours after cessation of infusion.

Tirofiban (Aggrastat)

Tirofiban (Aggrastat) is a nonpeptide (peptidomimetic), small-molecule inhibitor of the IIb/IIIa receptor, which also binds to the RGD receptor site, similar to Eptifibatide.

Oral GPIIb/IIIa antagonists

Oral GPIIb/IIIa antagonists, such as orbofiban, sibrafiban, and xemilofiban, were associated with a surprising excess in mortality. Development of these drugs was stopped. They are not clinically available.

Pediatric considerations

Aspirin dose in children is generally 1-5 mg/kg daily, but there is variability in the dose required to inhibit platelet aggregation. The primary side effect of long-term aspirin therapy is bleeding, but it is rarely seen except in neonates who have slower clearance, patients with concurrent bleeding disorders, or children receiving anticoagulation therapy. There is also a theoretical risk of developing Reye syndrome in children with intercurrent influenza or varicella infection, but this complication usually is not seen unless the dose of aspirin is >40 mg/kg, which is a high dose necessary for an anti-inflammatory effect. Dipyridamole in doses of 2-5 mg/kg is an alternative to aspirin therapy. Although there is growing use of antiplatelet agents (eg, clopidogrel) that selectively inhibit ADP-induced platelet aggregation, these drugs have not been well studied in children.

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. Low-molecular weight heparins (LMWHs) are made from UFH through chemical and physical processes and have a mean 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 five 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 (Arixtra) is a synthetic pentasaccharide that binds to AT, leading to specific inactivation of factor Xa.

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 (HIT) is defined as the occurrence of thrombocytopenia and a positive test for heparin-associated antibodies in a patient treated with heparin. Arterial thromboembolism and VTE can result. Strict criteria for HIT include a platelet count decrease to <100,000/ μ L or of >50% from baseline. Less strict definitions for HIT are a platelet count decrease to <150,000/ μ L or by >30% from baseline. The clinical picture of HIT (ie, thrombosis and demonstration of heparin-associated antibodies) in patients treated with heparin can occur even with platelet counts remaining normal or, rarely, unchanged counts (termed *HIT without thrombocytopenia*). Classically, the onset of thrombocytopenia is between days 5 and 10 after the initiation of heparin therapy, but it can occur in <1 day if the patient had had heparin exposure within the preceding 100 days. The *4T-score* has been evaluated as a tool to aid in the likelihood assessment that a patient has HIT. The four T's are as follows: (i) degree of thrombocytopenia, (ii) timing of thrombocytopenia, (iii) new thrombosis during heparin therapy, and (iv) alternative reason is present for the thrombocytopenia. Each of these four components is ranked 0-2 based on defined criteria, and the total score allows for an assessment as HIT unlikely, moderate suspicion for HIT, or HIT likely. Confirmatory HIT antibody tests are heparin-platelet-factor-4 antibody (HIT-PF4) enzyme-linked immunosorbent assay (ELISA), heparin-induced platelet aggregation study, and heparin-induced serotonin release assay. The HIT-PF4 ELISA is the most sensitive assay, but the least specific. Many patients exposed to high doses of heparin, such as after cardiopulmonary bypass surgery, develop heparin HIT-PF4 antibodies, which often do not lead to thrombocytopenia or thrombosis and appear to be clinically irrelevant. The HIT-PF4 ELISA is the test most widely used for the diagnosis of HIT. The heparin-induced platelet aggregation test and heparin-induced serotonin release assay are functional assays and are more specific for the pathogenic antibodies that actually cause the clinical picture of HIT.

HIT most commonly occurs in the patient on UFH, but it also can develop on LMWH. Although it more commonly occurs with intravenous heparin therapy, it also can be seen

with subcutaneous dosing. In the patient with a moderate or high suspicion for HIT, heparin should be discontinued and alternative anticoagulants started. Alternative anticoagulants that can be used in HIT are the direct thrombin inhibitors bivalirudin and argatroban. Desirudin is another direct thrombin inhibitor, available as a subcutaneous preparation, but not FDA approved for HIT. Danaparoid also has been used in HIT, but it was not FDA approved for that indication and is unavailable in the United States. Fondaparinux has rarely been associated with HIT and is sometimes used in HIT, as HIT antibodies generally do not cross-react with the drug. A lack of systematic studies has precluded the authors of the 2012 ACCP guidelines from making recommendations about the use of fondaparinux in HIT. Fondaparinux is not FDA approved for HIT, but it is suggested as a therapeutic option by some experts.

VKAs should not be used before the platelet count has increased to 150,000/ μ L. The alternate anticoagulant drug should overlap with the VKA for a minimum of 5 days. Because there is cross-reactivity of the HIT-PF4 antibody between UFH and LMWH, the latter is not a treatment alternative when HIT on UFH has been diagnosed. Detailed recommendations for platelet count monitoring while on heparin and for the diagnosis and treatment of HIT are available in the 2012 ACCP guidelines.

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 >400–450 sec) at which extracorporeal circulation on heparin is thought to be safe from an anticoagulant point of view. Causes can be AT deficiency; increased heparin clearance; significantly low baseline aPTT, such as due to elevations of factor VIII and fibrinogen; or increased nonspecific heparin-binding proteins. Occasionally, AT concentrate is considered in the treatment of heparin resistance, but no detailed guidelines exist as to who should receive it, in what doses, and for how long.

Unfractionated heparin

UFH at therapeutic doses typically if monitored with the 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 0.3–0.7 U/mL. Optimally, a coagulation laboratory should provide clinicians with the therapeutic aPTT range for the reagent-instrument combination used in that laboratory. If a laboratory has not provided a therapeutic aPTT range for

aPTT determinations, then an aPTT ratio of 2.0–2.5 times the mean aPTT 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 method. Although this is an acceptable alternative, it is not known whether one over the other 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. Patients with renal failure may require less UFH to prolong their aPTT into the therapeutic range. 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 3–4 hours after discontinuation of heparin.

Weight-based heparin dosing nomograms achieve therapeutic aPTTs faster than other approaches to selecting an 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 or coagulation factor XII deficiency, anti-Xa levels need to be used for heparin monitoring. Neonates may require higher doses of heparin because the clearance is more rapid secondary to a large volume of distribution and they have lower AT levels. Long-term use of UFH leads to an increased risk of osteoporosis. There is also a potential risk of osteoporosis with long-term LMWH use, but the risk is less than with unfractionated heparin.

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 compared with unfractionated heparin, so that fixed or weight-adjusted dosing is possible without the need for routine anticoagulant laboratory monitoring. The peak plasma effect is reached 3–4 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 renally cleared, dose reduction and anti-Xa monitoring is needed 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 may be appropriate and consultation of the package insert for the individual LMWH being used appears advisable to determine FDA recommendations on dose. In severe renal impairment and dialysis dependence, unfractionated heparin should be chosen over LMWH. In respect to thromboprophylaxis, there is a strong negative correlation between total body weight and anti-Xa levels. The best (ie, most effective) dosing regimen for patients with more extreme body weights is not known. It may be appropriate to increase the LMWH dose for patients with morbid obesity (body mass index of >40 kg/m²). For full-dose LMWH use, dosing should be based on absolute body weight and anti-Xa monitoring generally is not necessary for patients weighing up to 150 kg. Anti-Xa monitoring and twice- (rather than once-) daily dosing can be considered in patients with morbid obesity.

An expected anti-Xa level for once-daily dosing is in the order of 1.0-2.0 U/mL, for twice-daily dosing it is 0.6-1.2 U/mL, obtained 3-4 hours after subcutaneous injection. 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. That being said, neither "high" nor "low" levels of anti-Xa activity have been well correlated with the risk of adverse clinical outcomes. Many neonates (especially preterm) have minimal subcutaneous tissue making injection impractical, so that intravenous anticoagulation with LMWH can be considered. In children treated with therapeutic doses of LMWH, routine monitoring with anti-factor Xa levels is advocated, particularly as LMWH therapy often is instituted in critically or chronically ill children.

Fondaparinux

Fondaparinux (Arixtra) is a synthetic pentasaccharide, is AT dependent, and consists of the five 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 because of a half-life of approximately 17 hours, 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 renal failure. Protamine does not bind to fondaparinux. Recombinant factor VIIa can be used in major bleeding associated with fondaparinux, but it is not clear whether it has any beneficial effect.

Thrombin inhibitors

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 anticoagulant effect. Several derivatives and recombinant products have been developed. Lepirudin (Refludan) is a recombinant hirudin consisting of 65 amino acids that is administered intravenously and monitored by the aPTT. A therapeutic aPTT range is considered to be 1.5-2.5 times the median of the laboratory's normal aPTT range. The drug is renally cleared and has a half-life of approximately 80 minutes. It should not be used in patients with renal impairment. It is FDA approved for HIT. The manufacturer discontinued production in May 2012, however. Desirudin (Iprivask) is also a 65 amino acid recombinant hirudin, administered subcutaneously. Peak plasma levels are reached 1-3 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 (Angiomax) 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 (PTCA), including patients undergoing PTCA who have HIT.

Argatroban (Novastan)

Argatroban (Novastan) 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, dosage 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 40-50 minutes. The drug can be started without the need for an initial bolus. The dosing is adjusted to an aPTT of 1.5-3 times the initial baseline value (not to exceed 100 seconds). It is FDA approved for 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 8-6). The half-lives of the vitamin K-dependent coagulation factors are 4-6 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 create a 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, dose requirement

VKAs are monitored with the prothrombin time (PT). Because results of the PT depend on the sensitivity of the PT reagent used in the laboratory, PT measurements are converted to an INR by a calculation that includes a reagent's sensitivity (international sensitivity index). Coumarin VKAs are metabolized by the cytochrome P450 enzyme complex, mostly the enzymes CYP2C9 and CYP1A2 (Figure 8-6). 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 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 typically yield equally reliable results as plasma-based INRs from phlebotomies (this may not be true for some patients with APLA syndrome; see the relevant section in this chapter). 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 vitamin K, such as 100-150 µg/d, has been shown to decrease INR fluctuations.

VKAs available

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 (Coumadin, Janatoven) has a half-life of 1-2.5 days, with a mean of approximately 40 hours.

The typical loading dose of warfarin in the hospitalized patient is 5 mg daily on day 1 and 2, with subsequent dosing based on the INR measurement after the first two doses. In children, this will equate to initial doses of 0.1-0.2 mg/kg. A frail or elderly patient, one who has been treated with prolonged antibiotics, has liver disease, or has undergone intestinal resection, will need a lower dose in the first few days. Some clinicians prefer using higher loading doses of 7.5-10 mg, particularly in a young, nutritionally repleted 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 20-30 mg per day. Genetic testing for polymorphisms of the CYP2C9

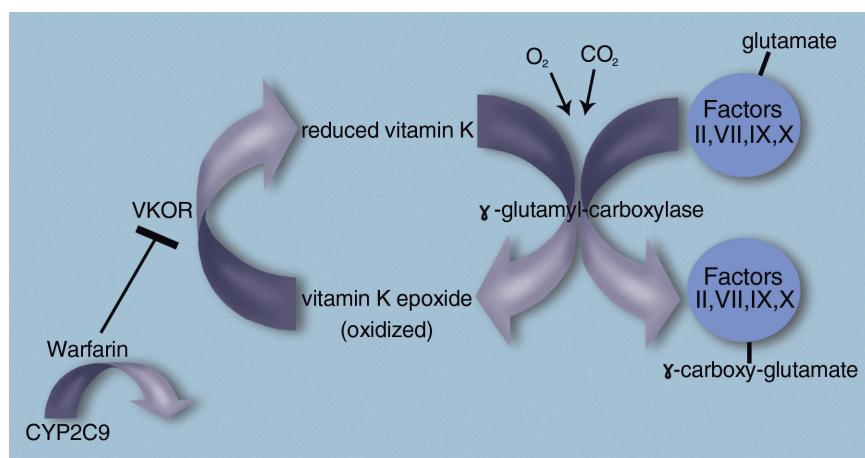


Figure 8-6 Role of vitamin K, point of activation of warfarin, and enzymes involved in vitamin K and warfarin metabolism.

Table 8-7 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
5.0-9.0	No	Yes	Vitamin K 1-2.5 mg p.o.
> 9.0	No	No/yes	Vitamin K 2.5-5 mg p.o.
Serious bleed at any INR	Yes		Vitamin K 10 mg i.v. + FFP or PCCs or recombinant factor VIIa

FFP = fresh frozen plasma; INR = international normalized ratio; PCCs = prothrombin complex concentrates; VKA = vitamin K antagonist.

and VKORC1 enzyme genes is available and helps predict, to some degree, warfarin doses needed to reach therapeutic INR ranges, but the clinical utility of these tests has not been clearly demonstrated.

Management of elevated INRs and bleeding

Several options exist to manage elevated INRs and bleeding that occurs on VKAs, and depend 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 8-7 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 will reverse the INR completely and may make reanticoagulation of the patient more difficult. FFP can lower the INR some but not completely or markedly because the amount of any particular clotting factor in a unit of plasma is small; thus, large and clinically impractical doses of FFP would have to be given for full reversal. If complete or immediate INR reversal is needed, such as when treating a major bleeding episode, a prothrombin complex concentrate (PCC) can be given. PCCs are plasma products from human donors and consist of the vitamin K-dependent factors (ie, II, VII, IX, and X). They exist as so-called *four-factor PCCs*, containing all vitamin K-dependent coagulation factors, and as *three-factor*

PCCs (eg, Bebulin, Profilnine), which contain relatively low concentrations of factor VII. The four-factor products are capable of restoring individual clotting factor activity to nearly 100% within minutes of administration of a low-volume intravenous infusion. No four-factor PCC is FDA approved or available in the United States as of March 2013. Therefore, in the United States, if a patient is being treated with three-factor PCCs for VKA-associated major bleeding, additional FFP or a low dose of recombinant factor VIIa should be considered to increase the patient's plasma levels of factor VII.

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 soon before the procedure to stop VKAs depends on the INR and the half-life of the drug used. Whether bridging therapy with a subcutaneous or intravenous anticoagulant needs to be given before and after the procedure depends on the thromboembolic risk of the patient (Table 8-8).

Pediatric considerations

Warfarin is the most commonly used VKA in children. Age is an independent predictor of warfarin dose, more so than the VKORC1 and CYP2C9 genotypes. Children ≤1 year of age

Table 8-8 Recommendations when interrupting warfarin therapy for invasive procedures.*

Risk of thrombosis	Before surgery	After surgery
Low	<ul style="list-style-type: none"> d/c warfarin ca. 5 d pre-op No LMWH or low dose LMWH 	<ul style="list-style-type: none"> Restart warfarin 12-24 hrs after surgery No LMWH or low dose LMWH
Intermediate	<ul style="list-style-type: none"> d/c warfarin ca. 5 d pre-op Prophylactic or full dose LMWH, or full-dose i.v. UFH 	<ul style="list-style-type: none"> Restart warfarin 12-24 hrs after surgery Prophylactic or full dose LMWH, or full-dose i.v. UFH
High	<ul style="list-style-type: none"> d/c warfarin ca. 5 d pre-op Full-dose LMWH or i.v. UFH 	<ul style="list-style-type: none"> Restart warfarin 12-24 hrs after surgery Full-dose LMWH or i.v. UFH

*These recommendations are all so-called “grade C” recommendations (ie, very weak recommendations). Other alternatives may be equally reasonable. d/c = discontinue.

require higher warfarin doses, longer overlap with heparin, longer time to achieve target INR ranges, and more frequent INR testing and dose adjustments, and they have fewer INR values in the target range. Other factors that affect VKA therapy in children include diet, drugs (specifically antibiotics), infections, underlying disease state, and weight gain. Because newborns have reduced levels of the vitamin K-dependent proteins, VKA therapy is challenging in very young children. Infant formulas containing vitamin K supplements can cause resistance to VKAs, whereas breastfed infants will be sensitive to VKAs, as there is negligible amount of vitamin K in breast milk. Infants and even some older children have inadequate venous access for frequent INR monitoring. The major complication of VKA use in children is bleeding. Reported non-hemorrhagic complications in children treated with VKA therapy for >1 year include hair loss, tracheal calcification, and loss of bone density. Point-of-care whole blood monitors have made regulating VKA therapy more convenient for families as they can perform the test regularly at home.

New oral anticoagulants

Several new oral anticoagulant drugs have been developed. 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) a wide therapeutic index that eliminates the need for routine monitoring of their anticoagulant effect in most patients; (iii) relatively few clinically important interactions with medications; (iv) no dietary restrictions; and (v) short half-lives that make perioperative anticoagulation management simpler by eliminating the need for “bridging therapy.” On the other hand, the dependence of some of these drugs on renal clearance limits their use in some patients. Some of these drugs are FDA approved and available for certain indications, some are awaiting FDA approval, and some are still undergoing clinical testing. As the approved indications are relatively rapidly expanding 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 four new oral anticoagulants furthest along in development are listed in Table 8-9, and include dabigatran (Pradaxa), rivaroxaban (Xarelto), apixaban (Eliquis), and edoxaban (Lixiana).

Atrial fibrillation

Large phase III randomized controlled trials have demonstrated that dabigatran, rivaroxaban, and apixaban are at

Table 8-9 New oral anticoagulants: selected pharmacologic properties and approval status.

Generic name	apixaban	dabigatran	edoxaban	rivaroxaban
Brand name	Eliquis®	Pradaxa®	Lixiana®	Xarelto®
Target	FXa	IIa	FXa	FXa
t-max (hours)	1-3	1.25-3	1-2	2-4
Half-life (hours) in patients with normal renal function	8-15	12-14	8-10	9-13
Effect of hepatic impairment	Mild-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
Renal excretion (%)	25	80	35-40	66
Effect of renal impairment	• CrCL 30–50: 1.29-fold greater exposure • CrCL 15–29: 1.44-fold greater exposure	• CrCL 30–50: 2.7-fold greater exposure • CrCL 10–30: 6-fold greater exposure (2-fold increase in the plasma half-life)	not reported	• CrCL 30–49: 1.5-fold greater exposure • CrCL 15–29: 1.6-fold greater exposure
Dosing frequency	Twice daily	Twice daily	Once daily	Once daily*
Drug interactions	CYP3A4/5	P-gp	P-gp	P-gp, CYP3A4
Approval status as of March 2013 (United States)	stroke prevention in AF	stroke prevention in AF	-	VTE prevention after total hip/knee arthroplasty; VTE treatment; stroke prevention in AF

*Rivaroxaban is given twice a day for the first 21 days in patients with acute VTE.

AF = atrial fibrillation, VTE = venous thromboembolism, CrCL = creatinine clearance (mL/min).

least as safe and effective as warfarin for the prevention of stroke in most patients with nonvalvular atrial fibrillation (AF). Dabigatran, rivaroxaban and apixaban have been FDA approved for the prevention of stroke in patients with nonvalvular AF since 2010, 2011, and 2012, respectively.

VTE prevention

Apixaban, dabigatran, rivaroxaban, and edoxaban have been studied for the prevention of VTE after total hip or knee arthroplasty and are approved for these indications in various jurisdictions. In the United States, as of March 2013, only rivaroxaban was FDA approved for these indications.

VTE treatment

The new oral anticoagulants have been compared with VKA therapy for the treatment of patients with acute VTE. As of March 2013, the data from the dabigatran and rivaroxaban studies have been published, the apixaban study is completed yet not published, and the edoxaban study is ongoing. Rivaroxaban is approved in the United States for acute treatment of VTE and for the secondary prevention of recurrent VTE.

Management issues

Several issues are important when managing patients who are being treated with these new oral anticoagulants. First, although routine monitoring of the anticoagulant effect of these drugs is not necessary, certain clinical situations may make measurement of their anticoagulant effect desirable, such as when investigating whether a patient who bleeds on one of the drugs is over-anticoagulated, or whether a patient who develops a clot is under-anticoagulated; when determining whether residual drug effect is present in the patient's plasma before a surgery that poses a high risk of bleeding; when wishing to test a patient's compliance with taking the prescribed drug; or when dosing the drugs in unusual patient populations (ie, those with renal or hepatic impairment or extreme body weights). Data on expected therapeutic plasma drug levels determined by clinical bleeding and clotting events and the performance of the various coagulation tests are beginning to be published. The clinician will need to keep updated on these data as they become available to use and interpret them appropriately for clinical care. Second, how long before surgeries to interrupt these new oral anticoagulants, which are cleared to a not insignificant degree by the kidney, depends on an individual's renal function, as this determines the drug's half-life; it also depends on the risk of bleeding with the particular surgery planned. Third, major bleeding on these drugs will occur. As of March 2013, no reversal agents were available and no reversal strategies had

been clinically tested and published. The hematologist will be called to assist with the management of patients with major bleeding. Therapy with oral charcoal is appropriate in the patient who ingested drug within 2 hours of presentation with major bleed. Further management is supportive. Dabigatran is dialyzable; rivaroxaban and apixaban, given their high plasma protein-binding, are not. Whether treatment with PCCs or recombinant VIIa (Novoseven) is clinically beneficial is not known. It is also not known whether anti-fibrinolytic therapy with tranexamic acid (Lysteda) or amino-caproic acid (Amicar) would have any beneficial effect in cases of severe bleeding. FFP would not be expected to have any efficacy. Fourth, when using the drugs for approved or nonapproved indications, the clinician needs to be clear about the differences between the drugs regarding their dosing (once versus twice daily), as well as the needed dose adjustment or avoidance of the drug in renally or hepatically impaired patients. Regarding their use in the acute treatment of VTE, it is important to realize that some of these drugs were used immediately upon the diagnosis of an acute VTE (rivaroxaban, apixaban), whereas others were given to the patient only after an initial few days of treatment with a parenteral anticoagulant (dabigatran, edoxaban). Few patients with antiphospholipid syndrome, cancer, or warfarin failure were included in the VTE treatment trials of these new drugs; thus, they should be used with caution in these subpopulations, even after approval for use in unselected patients with VTE.

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 8-5). In clinical practice, these drugs are used in various FDA-approved and non-FDA-approved indications for the management of thrombotic clinical disorders. Streptokinase is derived from the culture of beta-hemolytic streptococci and urokinase is derived from the tissue culture of human neonatal kidney cells. Alteplase (Cathflow, Activase) 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 (Retavase) is such a mutant tPA molecule, modified to be only 355 amino acids long. This leads to a longer half-life and better penetrance into clots. Tenectaplate (TNKase) is a recombinant full-length tPA molecule with three 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). In neonates and children <12 months of age, there is a need for plasminogen supplementation (using FFP) before administration of tPA, as plasma concentrations of plasminogen in the first year of life are 50% lower than adults, making thrombolytic therapy less effective.

Venous thromboembolism

VTE is inconsistently defined. For some, it encompasses any thrombosis in veins (no matter whether superficial or deep veins) plus pulmonary embolism; for others, it only signifies DVT and PE. Estimates suggest that at least 300,000, and as many as 600,000 people, in the United States develop DVT/PE each year, and that at least 60,000-100,000 deaths each year are due to DVT/PE. Approximately half of DVT/PE episodes are hospital associated. VTE in children is uncommon, but hospitalization increases the risk: DVT/PE occurs in 1 in 200-300 hospitalized children. There is a bimodal peak in infants and children, with the highest rates found in neonates and adolescents.

Superficial thrombophlebitis

Superficial thrombophlebitis may occur unprovoked and unexplained (also called idiopathic), or in the setting of varicose veins, trauma, intravenous catheters or phlebotomy, underlying hypercoagulable states, cancer, or as septic thrombophlebitis with infections. It also occurs in association with inflammatory bowel disease, thrombangiitis obliterans (Buerger's disease), and Behçet's disease.

Superficial thrombophlebitis typically has a benign course. Thrombophlebitis that is not very extensive (ie, less than 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. Patients with extensive or recalcitrant superficial thrombophlebitis may benefit from a short course of out-of-hospital anticoagulant therapy, such as up to 6 weeks of subcutaneously administered unfractionated heparin, LMWH, or fondaparinux. Both the optimal dosing of these drugs (full dose, intermediate dose, or prophylactic low dose) and the duration of therapy are not well defined; 45 days of fondaparinux, in comparison to conservative treatment, has been shown to reduce the risk of DVT extension, but the number needed to treat (to prevent one clinically important event) is very large and the cost effectiveness of this strategy is debated. Extension of superficial thrombophlebitis into the deep venous system occurs in about 1 in 6 patients with extensive superficial thrombophlebitis. To rule out extension, ultrasonography should be considered in all patients with extensive superficial thrombophlebitis.

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. It sometimes is used for any venous thrombosis (ie, superficial thrombophlebitis, DVT, or PE) occurring in patients with known or yet unknown cancer, particularly when the thrombotic events are recurrent. Mondor's disease is the term used for thrombophlebitis of the superficial veins of the breast and anterior chest wall, typically occurring after breast cancer surgery and mammoplasties.

Deep vein thrombosis and pulmonary embolism

Prevention

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 DVT prophylaxis guidelines should be in use in all hospitals.

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 intensely than anticoagulant-based methods. Although there is some evidence that aspirin and other antiplatelet agents provide some protection against VTE in hospitalized patients at risk, they are probably inferior to other pharmacologic methods of VTE prophylaxis.

The mainstay of VTE prophylaxis is anticoagulant drugs. Several options are available: (i) unfractionated heparin at every 8- or 12-hour dosing intervals; (ii) LMWHs at once- or twice-daily intervals; (iii) fondaparinux once daily; (iv) VKAs; or (v) one of the new oral anticoagulants. FDA-approved indications vary between the different pharmacological options. 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) is well established in patients after hip fracture, hip replacement, and major cancer surgery. Data from other clinical settings support a conservative approach to posthospitalization prophylaxis for most patients.

Symptoms

DVT of the pelvic and leg veins presents with varying degrees of leg swelling, pain, warmth, and skin discoloration. Symptoms are typically diffuse. Localized symptoms are more

suggestive of a superficial thrombophlebitis. A palpable subcutaneous cord-like firmness is indicative of a superficial thrombophlebitis. The onset of symptoms of DVT can be sudden or subacute over days to weeks. DVT is not infrequently 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 often are asymptomatic. 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.

Diagnosis

Scoring systems based on a patient's VTE risk factors and clinical symptoms and findings have been established to determine how likely it is that a patient presenting with leg or lung symptoms has DVT or PE. The grouping into low and intermediate or high pretest probability helps guide which further diagnostic tests to perform. The whole blood or plasma D-dimer tests are well evaluated and useful in the diagnostic work-up 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 rules out 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. 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, 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 unsafe, unless the test has been validated locally. In children, the D-dimer test as a diagnostic tool for VTE has not been well studied.

Venous Doppler ultrasound is the most widely used imaging study to look for DVT of the legs. Sensitivity and specificity of the test are operator dependent and an experienced ultrasound technician or physician is key in obtaining reliable results. 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 computer tomography (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.

To diagnose PE, several imaging modalities exist: ventilation/perfusion (VQ) scanning, PE-protocol chest CT angiography (also known as spiral CT, helical CT or PE-protocol CT), chest MR angiography, and conventional intravenous contrast pulmonary angiogram. The VQ scan is a well-validated imaging study. PE-protocol chest CTs have replaced VQ scans as the diagnostic method of choice, however, because they are easier and faster to perform and have good performance characteristics. Their predictive value with a concordant clinical assessment is high, but additional testing is necessary when the clinical probability is inconsistent with the imaging results. Conventional intravenous contrast pulmonary angiography, once considered the gold standard for the diagnosis of PE, now is rarely done, because the test is invasive, not widely available, and has diagnostic limitations. In the patient with PE and significant clinical symptoms or extensive clot on imaging study, an echocardiogram should be performed to assess for right ventricular dysfunction. Biologic serum markers, such as cardiac troponin and brain natriuretic peptide levels, are helpful in determining the degree of right-heart strain.

Acute therapy

Outpatient management of patients with DVT and PE has been shown to be safe, feasible, and cost effective and, if possible, is the preferred treatment of choice. Hospital admission is appropriate if the patient is too sick to be managed at home or if social and financial circumstances make this the safer and more feasible option.

Patients with acute VTE need to be anticoagulated to prevent the extension of thrombus and decrease mortality. Intravenous UFH as well as subcutaneous LMWH and fondaparinux are all effective and acceptable treatment options and need to be given for at least 5 days and until the INR is ≥ 2.0 for 24 hours. As of March 2013, rivaroxaban was the only one of the new oral anticoagulants approved by the FDA in the United States for the treatment of VTE. It can be given to patients with acute DVT or PE without the need for bridging with a parenteral anticoagulant. In young children, appropriate dosing and monitoring of fondaparinux has not been studied. In patients who are potentially unstable because of significant PE, UFH is preferable over LMWH or fondaparinux because it has a shorter half-life and easily can be dose-adjusted, discontinued, or reversed (with protamine) if bleeding occurs or thrombotic therapy has to be given. In selected patients with extensive acute proximal DVT with symptom duration of

<14 days and with low bleeding risk, catheter-directed thrombolysis with or without mechanical thrombus fragmentation and aspiration can be considered to reduce acute symptoms and potentially to decrease the risk of developing postthrombotic syndrome. Whether thrombolytic therapy with or without mechanical thrombectomy decreases the incidence or severity of postthrombotic syndrome has not yet been demonstrated.

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 a May-Thurner syndrome is demonstrated on venography or MR imaging in the patient with left-leg proximal DVT who successfully has received thrombolytic therapy, correction of the stenosis using balloon angioplasty and stenting can be considered.

Thrombolytic therapy in PE is indicated for massive life-threatening PE (ie, PE with hypotension). Selected high-risk PE patients without hypotension and at low risk for bleeding may receive thrombolytics. In a prospective randomized trial in patients with submassive PE, however, thrombolytic therapy did not decrease mortality compared with no thrombolytic therapy. It is not known whether thrombolytic therapy decreases the long-term risk of pulmonary hypertension. Although thrombolytic therapy does carry a risk of severe bleeding, in carefully selected patients, it has been shown to be relatively safe and not lead to more serious bleeding events than treatment with UFH alone. 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. If no thrombolytics are given, oral VKAs or rivaroxaban can be started on the day of presentation. Educating the patient about VTE and anticoagulant therapy with all its facets is important.

Duration of anticoagulant therapy

The risk of recurrent VTE in patients with a VTE secondary to a major transient (reversible) risk factor is low. Therefore, time-limited anticoagulation for 3 months with a VKA is recommended. For patients with unprovoked proximal DVT as their first VTE and in whom risk factors for bleeding are absent and for whom good anticoagulation control is achievable, consideration of long-term (extended) VKA therapy is recommended by the 2012 ACCP guidelines. In clinical practice, however, this is a complex issue. Several parameters can be used in the discussion with the patient about the individual risk of recurrence (Table 8-10) and a decision about continuation or discontinuation can be made based on patient-individual risk factors for VTE recurrence

Table 8-10 Considerations when discussing time-limited versus long-term anticoagulation therapy in patients with unprovoked VTE.

Reasons contributing to a decision for long-term VKA therapy

- Recurrent VTE
- Strong thrombophilia present (ie, APLA syndrome, antithrombin deficiency, protein C or S deficiency, homozygous factor V Leiden, double heterozygous state for factor V Leiden and prothrombin 20210 mutation)
- Male gender
- Patient had a PE ± DVT, not just a DVT
- D-dimer result on VKA therapy positive at 3 or 6 months
- D-dimer positive after having been off VKA for 4 weeks
- VKA well tolerated with good control of INR and no bleeding complications
- Little or no impact of anticoagulant therapy on patient's lifestyle (profession, hobbies)
- Patient's preference is to stay on VKA

Reasons contributing to a decision against long-term VKA therapy

- VTE was associated with estrogen excess (estrogen contraceptives, hormone replacement therapy, pregnancy)
- Female gender
- Distal DVT only
- D-dimer result on VKA therapy negative at 3 or 6 months
- D-dimer negative after having been off VKA for 4 weeks
- VKA poorly tolerated with widely fluctuating INRs
- Occurrence of bleeding complications or significant risk for bleeding
- Significant impact of anticoagulant therapy on patient's lifestyle
- Patient's preference is to come off VKA

APLA = antiphospholipid antibody; DVT = deep vein thrombosis; INR = international normalized ratio; PE = pulmonary embolism; VKA = vitamin K antagonist; VTE = venous thromboembolism.

and bleeding as well as patient preference (Figure 8-7). When extended anticoagulation therapy is chosen, the risks, benefits, and burdens of long-term anticoagulant therapy should be reevaluated periodically (eg, once a year). The popliteal vein is considered a proximal vein. Thrombus in a calf vein (ie, in one of the veins after the trifurcation) is considered a distal leg DVT. In patients with distal DVT without severe symptoms, serial follow-up Doppler ultrasound imaging of the leg veins over 2 weeks is suggested by the 2012 ACCP guidelines, rather than anticoagulation therapy. This is a grade 2C recommendation (ie, a weak recommendation based on low- or very low-quality evidence). For patients with a first episode of unprovoked distal leg DVT with severe symptoms, 3 months of VKA therapy is recommended.

To aid decision making in which patients to discontinue and in which to continue anticoagulation, risk scores (eg, HERDOO-2 score, DASH score) have been created based on data from VTE trials in which the rate of recurrent VTE

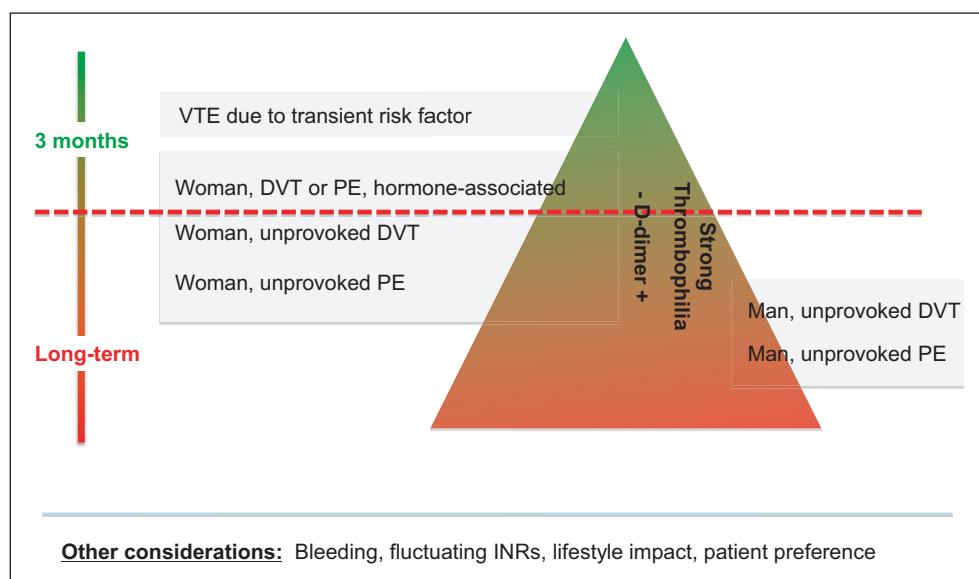


Figure 8-7 Management strategy regarding length of anti-coagulation therapy decisions in patients after a first episode of provoked or unprovoked proximal VTE. DVT = deep venous thrombosis; INR = international normalized ratio; PE = pulmonary embolism.

was recorded and subgroup analyses were performed to identify predictors of recurrent VTE. Neither score is ready for routine clinical use, however, as both still need to be validated in prospective clinical trials. Per HERDOO-2 score, being male always indicates a higher risk of recurrence; in women, the presence of postthrombotic syndrome, a positive D-dimer while taking warfarin, a body mass index of $>30 \text{ kg/m}^2$, and age >70 years indicate a higher risk of recurrence. Per DASH score, a higher risk of recurrence is predicted by a positive D-dimer after discontinuation of anticoagulation, age <50 years, male gender, and the fact that the first VTE event was not associated by hormone use.

In most patients, a target INR of 2.0-3.0 is appropriate and protects to a large degree from recurrent VTE. In patients with cancer, the risk of recurrent VTE on VKA is high, even if the INR is in the 2.0-3.0 range. LMWH has been shown to be more effective than VKAs in preventing recurrences in these patients and is the preferred treatment, if feasible, from a financial and insurance reimbursement point of view. If recurrent VTE occurs in spite of documented therapeutic INRs, anticoagulant treatment options are either to continue VKA but increase the target INR or to switch to another anticoagulant, such as long-term LMWH or fondaparinux. In one prospective, randomized trial (WARFASA study), aspirin was shown to have benefit in the prevention of recurrent VTE in patients with previous unprovoked VTE, although the benefit was much less than that expected from long-term anticoagulant secondary VTE prophylaxis. A second study of similar design (ASPIRE study) did not show a benefit of aspirin in the prevention of recurrent VTE. Thus, at this point, the role of aspirin in the secondary prevention of VTE is not clear.

Pediatric considerations

The current management of acute VTE in children is extrapolated from adult patients. LMWH is the preferred anticoagulant in children for the treatment of VTE because of its predictable pharmacokinetics and lack of interference with diet. 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. The 2012 ACCP guidelines provide details on dosing regimens and monitoring for specific anticoagulants. The majority of VTE events in children are caused by transient risk factors, such as central venous catheters, and these children are treated for 6 weeks to 3 months with anticoagulation. The 2012 ACCP guidelines suggest that children with unprovoked (idiopathic) VTE receive anticoagulant therapy for 6-12 months. Children with inherited higher risk thrombophilias are treated like adult patients and receive long-term anticoagulation depending on their risk of recurrence.

Postthrombotic syndrome

Postthrombotic syndrome is caused by an interplay between incompetent venous valves damaged by the thrombus or associated inflammatory mediators and impairment of venous return due to residual venous obstruction from incompletely cleared thrombus. Approximately a third to half of DVT patients will develop postthrombotic syndrome (sometimes referred to as postphlebitic syndrome), in most cases within 1-2 years of the acute DVT. Symptoms and signs include chronic extremity swelling, pain, heaviness, fatigue, paresthesias, skin induration, dryness, pruritus, erythema and chronic

dark pigmentation, and, in more severe cases, skin ulcers. The risk for developing postthrombotic syndrome has been thought to be decreased if graduated compression stockings (40 mm at ankle, 30 mm at mid calf) are worn for 2 year after the acute DVT, but a recent randomized trial, the SOX trial, has questioned that view. Treatment options for patients with postthrombotic syndrome are limited. Compression stockings should be worn. 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 that might be amenable to pelvic vein angioplasty and stenting. Also, a home compression pump with compression sleeve for the affected leg should be considered for patients with significant symptoms. Similar to adults, almost 30% of children are diagnosed with postthrombotic syndrome. 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 and occurs in 1% of patients with acute PE after 6 months, 3.1% after 1 year, and 3.8% after 2 years. CTEPH can be the result of only one event of PE in the past in which the PE did not resolve appropriately or the result of recurrent episodes of PE. The patient who experiences chronic shortness or significant generalized malaise after a large PE should be evaluated for pulmonary hypertension. A formal 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 VQ scan is the screening imaging test of choice and is indicated. Right-heart catheterization with pulmonary artery pressure measurements then defines the degree of hypertension, and pulmonary arteriography helps define the etiology and allows assessment of whether potentially curative pulmonary endarterectomy is indicated. 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 or the one in whom surgery did not result in improvement.

Inferior vena cava filters

A clear indication for an inferior vena cava (IVC) filter exists only when a patient has acute pelvic or proximal leg DVT but cannot be anticoagulated because of active

bleeding or very high bleeding risk. It is not clear whether an IVC filter is beneficial in the patient with recurrent pelvic or proximal leg DVT in spite of therapeutic anticoagulation. The 2012 ACCP guidelines suggest that IVC filters not be used for primary VTE prevention in patients with trauma or undergoing major abdominal or pelvic surgery. When IVC filters are placed, retrievable filters should be considered, unless the intention at the time of placement is that they stay in permanently. Retrievable filters can be left in place for weeks to months or can remain permanently, if necessary. For patients with acute VTE who have an IVC filter inserted as an alternative to anticoagulation, it is recommended that a conventional course of anticoagulant therapy be given once the patient's risk of bleeding has resolved. Presence of a permanent IVC filter increases a patient's risk for recurrent DVT. 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 one of the risk factors for recurrent VTE. A decision as to whether to continue anticoagulants should be made based on the sum of all prothrombotic risk factors, balanced against the risk factors for bleeding, as well as the implications that long-term anticoagulation may have on a patient's quality of life.

Venous stents

Venous stents may be placed in various locations of the venous system, most commonly into the left common iliac vein due to May-Thurner syndrome, the right and left pelvic veins due to postthrombotic vessel narrowing and scarring, and the superior vena cava and central arm veins in central venous catheter-associated strictures. The best long-term management of patients who have venous stents is not known due to a lack of 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 to make an assessment on need for long-term anticoagulation versus no further anticoagulation based on a comprehensive assessment of the patient's risk factors for recurrent VTE and bleeding. It is not known whether a patient who has a venous stent but is not on long-term anticoagulants will benefit from long-term antiplatelet therapy.

Unusual venous thromboses

Upper-extremity DVT

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%-4% of all DVT. Roughly 80% are secondary to central venous catheters and cancer, and 20% are primary events; however, these data depend largely on what patient population is studied. Doppler ultrasound (sensitivity 78%-100% and specificity 82%-100%), contrast venography (gold standard), and MR venography are the diagnostic tools used to diagnose upper-extremity thrombosis. PE occurs in 2%-35% of patients. Postthrombotic syndrome is frequent, occurring in 7%-46% of patients, and residual thrombosis and axillo-subclavian vein thrombosis appear to be associated with a higher risk, whereas catheter-associated DVT may be associated with a lower risk.

Management for DVT of the upper-extremity consists of the following: (i) LMWH, UFH, or fondaparinux in the acute setting; (ii) VKA or rivaroxaban for 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. 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. It is noteworthy that there is little or no direct evidence to support any particular duration of anticoagulant therapy after a first unprovoked 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. There is no uniform approach to treatment of these patients. Management options include anticoagulation, thrombolytic therapy, angioplasty, 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 vascular interventional radiology may be appropriate.

Catheter-related thrombosis

Catheter-related thrombosis can be of several types: (i) fibrin sleeves, (ii) catheter tip thrombosis, and (iii) catheter-related

DVT. The first two may lead to catheter malfunction with inability to infuse or withdraw blood and fluids. tPA is FDA approved for this indication and an instillation of 2 mg into the catheter leads to catheter clearance in 75% of cases. The cumulative patency rate after a second dose is 85%. In children and neonates as many as 60% and 90% of thrombotic events, respectively, are catheter-associated. Thromboprophylaxis is not recommended routinely in patients with central venous catheters, as recent large and well-designed studies have not demonstrated efficacy of low-dose warfarin, LMWH, or aspirin in preventing catheter-associated DVT.

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 one thrombotic risk factor, and many (46%) have more than one risk factor; the most common are MPDs (49% of patients). P vera accounts for 27% percent of cases; ET and 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 MPD are present. This is discussed in detail in the section "Thrombophilia." Any of the inherited and acquired thrombophilias can contribute to the development of Budd-Chiari syndrome, as can estrogens and pregnancy. 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 magnetic resonance imaging (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 or fondaparinux instead of VKAs can be considered.

Portal vein thrombosis

Portal vein thrombosis 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 MPDs, *JAK2*-positive status without overt MPD, PNH, intra-abdominal neoplasia, infection, trauma, surgery, and neonatal umbilical vein catheterization. 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. Diagnosis typically is made by Doppler ultrasonography. CT or MR venography also can provide evidence that portal vein thrombosis is present. Cavernous transformation of the portal vein reflects old portal vein thrombosis, as do collaterals in the porta hepatis. In the patient with acute portal vein thrombosis, 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 portal vein thrombosis typically is anticoagulated for at least 3–6 months to prevent progression of thrombosis. Regarding long-term anticoagulation therapy in these patients, as well as in patients with incidentally discovered portal vein thrombosis, the risk of bleeding has to be balanced individually against the risk of rethrombosis. The net benefit of anticoagulation for a patient with asymptomatic, cirrhosis-associated portal vein thrombosis is uncertain. 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 of the risk of 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, therefore, leads mostly to small bowel ischemic changes. The very rare IMV thrombosis may lead to ischemia in the sigmoid colon. Mesenteric vein thrombosis may be caused by trauma, surgery, intra-abdominal infections, inflammatory bowel disease, pancreatic disease, and progression of portal vein thrombosis, but also may occur spontaneously, particularly in patients with inherited or acquired thrombophilias, MPDs, 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 mesenteric vein thrombosis 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 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 VKA therapy depend, as with most of the other VTE disorders, on the triggers for the thrombotic episode, and the presence of thrombophilias or other permanent risk factors. Length of treatment is at least 3 months but may have to be long term.

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 mesenteric vein thrombosis, 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. Length of anticoagulant treatment depends on the triggering factors and the persistent thrombophilic risk factors.

Cerebral and sinus vein thrombosis

Blood from the brain drains via cerebral and cortical veins into the dural sinuses, which then drain into the internal jugular veins. Thrombosis of the cerebral, cortical, and sinus veins often is referred to as *cerebral and sinus vein thrombosis* or *cerebral sinovenous thrombosis*. It is seen much more commonly in the neonatal period than in any other age-group. It occurs in 3–4 cases per 1 million in the general population, in up to 7 cases per 1 million in children, and in approximately 120 cases in the peri- and postpartum period per 1 million deliveries. Although the outcome is relatively good in adults, half of neonates die. As with other venous thromboembolic events, cerebral and sinus vein thrombosis often is multifactorial, and the inherited and acquired thrombophilias play a role in its etiology, as do estrogen therapy and pregnancy. Infections, such as mastoiditis, otitis, sinusitis, and meningitis are risk factors, and, in neonates, dehydration, and perinatal complications are contributors. A cause for cerebral and sinus vein thrombosis is identified in 85% of patients. In

adults, the most frequent but least specific symptom is severe headache, either of subacute or acute onset, present in 90% of patients. In children, seizures, focal neurological signs, and headache are the most common manifestations. Routine noncontrast and contrast head CT scans and brain MRI scan often are unrevealing, resulting in missed diagnoses, unless CT venogram or MR venogram are requested specifically.

Approximately 40% of patients with cerebral and sinus vein thrombosis have a hemorrhagic infarct, which is a consequence of the venous occlusion. Currently, no available evidence from randomized controlled trials exists regarding the efficacy or safety of systemic or local thrombolytic therapy in cerebral and sinus vein thrombosis. On this basis of limited evidence, anticoagulation with heparin appears to be safe, not increasing the risk for intracranial bleeding, but rather leading to a potentially important reduction in the risk of death or dependency as a result of neurological impairment. This reduction, however, did not reach statistical significance. Therefore, heparin often is used in acute cerebral and sinus vein thrombosis, even if some parenchymal hemorrhage is present. The 2012 ACCP guidelines do not make any reference to anticoagulant management of patients with cerebral and sinus vein thrombosis, but the 2006 European Federation of Neurological Societies recommend LMWH or UFH therapy in the acute treatment. The optimal duration of anticoagulant therapy is unknown. Usually, VKAs are given after a first episode of cerebral and sinus vein thrombosis for 3 months if the thrombosis was associated with a transient risk factor, for 6–12 months if the event was unexplained and no higher risk thrombophilia has been detected, and long term if a higher risk thrombophilia is detected or the event is recurrent. This is based on expert opinion (eg, professionals from the American Heart Association, American Stroke Association, and others) and not on high-quality evidence, but it is supported by the observation that the recurrence rate of cerebral and sinus vein thrombosis is low.

In children, use of anticoagulant therapy with cerebral and sinus vein thrombosis has not been well studied in clinical trials, but it is not associated with serious hemorrhage in selected patients.

Renal vein thrombosis

In adults, the classical symptom triad of acute renal vein thrombosis, namely, acute flank pain, hematuria, and sudden deterioration of renal function, is seen only uncommonly. 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%–50% of patients with chronic nephrotic syndrome have evidence of renal vein thrombosis, 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 by 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 on whether the thrombotic event was associated with a transient prothrombotic risk factor, whether the patient has permanent risk factors or a higher risk thrombophilia. Renal vein thrombosis in children typically presents within the first month of life. In neonates, it may be associated with acute dehydration, perinatal asphyxia, and polycythemia. Infants of diabetic mothers are particularly at risk for renal vein thrombosis. Outside of the neonatal period, nephrotic syndrome is the most common risk factor for thrombosis in children as well as in adults.

Retinal vein thrombosis

Thrombosis can occur as central retinal vein occlusion (CRVO) or as branch retinal vein occlusion (BRVO). Unfortunately, clinical studies have often grouped CRVO and BRVO together, so that a differential effect of thrombophilia on CRVO and BRVO may have gotten lost in these studies.

CRVO has a prevalence of 1 in 250 to 1,000 in individuals ≥40 years of age. A number of studies on the association of various thrombophilias and CRVO have shown conflicting results; some have shown associations but most have not. The presence of cardiovascular risk factors, such as hypertension, hyperlipidemia, and diabetes, has been associated with CRVO. Unfortunately, there is a lack of randomized treatment trials investigating the usefulness of antiplatelet or anticoagulant therapy. The result is a lack of knowledge concerning the appropriate treatment for CRVO in facilitating clot resolution, symptom improvement, or prevention of recurrences.

BRVO is more common than CRVO and typically is the result of pressure on the vein from the overlying branch retinal artery, typically due to arteriosclerosis of the retinal artery. BRVO causes a painless sectoral decrease in vision, resulting in misty or distorted vision. Findings are those of intraretinal hemorrhage, retinal exudates, retinal ischemia, and macular edema.

Arterial thromboembolism

General comments

The hematologist typically does not get called on for input into the management of patients with ischemic disease that is due to arteriosclerosis. This is more the domain of the

cardiologist, neurologist, general internist, and endocrinologist. 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. Because the hematologist may be consulted for some antithrombotic management issues, a few key points on antithrombotic drug use in these disease states are made in the following sections. The more classical reason for consultation about a patient with arterial thromboembolism is when the event occurred in a person who is young, has no significant arteriosclerosis risk factors, or has a personal or family history of thrombophilia.

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 8-11). 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 in the young, spontaneous or traumatic cervical artery dissection should be considered.

Relatively little is known about thrombophilias predisposing to arterial thrombosis. Heterozygous FVL and heterozygous prothrombin 20210 mutation by themselves do not clearly increase the risk for arterial thromboembolism. It is not known whether the risk is increased in the individual with homozygous FVL or with homozygous prothrombin 20210 mutation, or in double heterozygous individuals with both FVL and the prothrombin 20210 mutation. Whether such individuals, as well as those with arterial thromboembolic events associated with deficiencies in protein C, protein S, and AT, should be treated with antiplatelet therapy or anti-coagulant therapy also is not known.

Peripheral arterial disease

Detailed recommendations for anticoagulant and antiplatelet management of patients with atrial fibrillation are available, such as the 2012 ACCP guidelines and the 2011 American Heart Association and American College of Cardiology guidelines (Table 8-12). Indications for antiplatelet

guidelines (Table 8-12). For individuals with symptomatic lower-extremity peripheral arterial disease, antiplatelet therapy is indicated, such as aspirin in daily doses of 75-100 mg. Clopidogrel (Plavix®) is an effective alternative. In patients with moderate to severe claudication, cilostazol (Pletal®) at 100 gm every 12 hours is indicated in the absence of heart failure, to improve symptoms and walking distance. There is no indication for the use of VKAs. The clinical effectiveness of pentoxifylline (Trental®) at 400 mg three times per day is marginal and not well established. The 2012 ACCP guidelines, therefore, recommend against its use. Patients with an acute ischemic thromboembolic event should be treated with UFH. In patients undergoing embolectomy, VKA for some period of time should be given. For patients with infrainguinal bypass, aspirin, but not routine treatment with VKAs, is indicated. For patients at high risk of bypass occlusion and limb loss, aspirin plus VKA is recommended. Patients undergoing carotid endarterectomy should receive preoperative aspirin at a dose of 75-100 mg. Long-term aspirin, 75-100 mg per day, should be given to patients after endarterectomy, with asymptomatic or recurrent carotid stenosis, and those undergoing extremity balloon angioplasty.

Pediatric consideration

Indwelling arterial catheters are widely used in neonatal and pediatric intensive care units for hemodynamic monitoring and blood sampling purposes. Insertion through the umbilical artery often is used for preterm and term neonates, whereas in infants and older children the preferred site is the radial artery. Alternative insertion sites include the ulnar, brachial, axillary, dorsalis pedis, and tibialis posterior arteries. The most frequent complication is development of arterial thrombosis. The overall incidence of indwelling arterial catheter related thrombosis is approximately 3.2%; 90% of these events occur in the femoral artery. The most serious complications of arterial thrombosis include loss of limb, organ, or life. Close clinical monitoring for skin discoloration, loss of pulses distal to the catheter and organ dysfunction is required. Depending on the severity of the complications, management ranges from removal of arterial catheters to the use of thrombolytic therapy followed by standard anticoagulation. Neonates with umbilical artery catheters should be monitored for extension of thrombosis to renal vessels.

Atrial fibrillation and stroke prevention

Detailed recommendations for anticoagulant and antiplatelet management of patients with atrial fibrillation are available, such as the 2012 ACCP guidelines and the 2011 American Heart Association and American College of Cardiology guidelines (Table 8-12). Indications for antiplatelet

Table 8-11 “Unexplained” arterial thromboembolism: suggested approach to structured evaluation.

A. Is arteriosclerosis the underlying problem?

- Arteriosclerotic changes demonstrated on imaging studies (on CT, contrast arteriogram or other radiologic imaging studies, on 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 cocaine or anabolic steroids?
- Is there evidence for Buerger’s disease (does patient smoke or use 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 work-up for vasculitis and immune disorder.
- Is there a suggestion of an infectious arteritis?
- Could the patient have hyperviscosity or cryoglobulins?

D. Thrombophilia work-up

- Hemoglobin and platelet count
- Antiphospholipid antibodies
 - Anticardiolipin IgG and IgM antibodies
 - Anti- β_2 -glycoprotein-I IgG and IgM antibodies
 - Lupus anticoagulant
- Protein C activity*
- Protein S activity and free protein S antigen*
- Antithrombin activity*
- Homocysteine
- Factor V Leiden and prothrombin 20210 mutation (purpose of testing is to detect the homozygous or double heterozygous state*)
- Do not test for MTHFR polymorphisms, PAI-1 or tPA levels or polymorphisms, fibrinogen or factor VIII activities.

* Uncertain clinical utility.

EKG = electrocardiogram; PAI-1 = plasminogen activator inhibitor-1; tPA = tissue plasminogen activator.

or anticoagulant therapy are based on the CHADS₂ score, a clinical prediction rule for estimating the risk of stroke in patients with nonrheumatic atrial fibrillation. The CHADS₂ score is made up of five risk factors for stroke in patients with atrial fibrillation: “C” for congestive heart failure (1 point), “H” for hypertension (1 point), “A” for age >75 years (1 point), “D” for diabetes (1 point), and “S₂” for prior stroke or TIA (2 points). The total number of points in an individual patient with atrial fibrillation predicts the risk for stroke and, thus, guides the choice of antithrombotic drug for stroke prevention: (i) score of 0: aspirin 81–325 mg or no antithrombotic therapy; (ii) score of 1: aspirin, no antithrombotic treatment,

or an anticoagulant; and (iii) score of ≥2: an anticoagulant. Most of the latest guidelines and opinions suggest either to anticoagulate or, in the case of patients with very low stroke risk, to do nothing. The results of the BAFTA trial and the AVERROES trial make it hard to rationalize aspirin as a stroke prevention strategy for any subgroup of atrial fibrillation patients.

Neonatal stroke

Neonatal stroke, defined as a cerebrovascular event that occurs between 28 weeks gestation and 7 days of age, occurs

Table 8-12 Key resources for use of antithrombotic drugs in arteriosclerotic occlusive arterial disease, atrial fibrillation and valvular heart disease.

	Antithrombotic therapy guidelines	
	ACCP 2012	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.

in 1 in 250 live births. There is a male predominance. Seizure is the most common clinical presentation. Many infants do not present for several months, however, until they are noted to have hemiparesis or early hand preference. 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 is the best test to determine extent of disease. There is no standard approach for the evaluation and treatment of perinatal stroke. 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, CMV) 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. Several studies have demonstrated an association between inherited prothrombotic conditions and neonatal stroke. The incidence of recurrent stroke is extremely low (less than 5%); therefore, anticoagulation is not indicated unless there is evidence of embolic heart disease. Children with a cardioembolic cause of stroke should be referred to a pediatric cardiologist for evaluation, management, and correction of the heart defect. Fifty percent of infants with perinatal events will be neurologically normal by 12-18 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.

Childhood stroke

Stroke affects 1 in 7,000 to 35,000 children per year, with a male predominance. As many as 65% of affected children will have 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.

Certain comorbid conditions are highly correlated with stroke, including congenital or acquired cardiac disease that can cause embolic phenomena and sickle cell anemia. Numerous systemic disorders can contribute to stroke, and inherited prothrombotic states are causative as well. A rare cause of stroke in childhood is severe iron deficiency anemia. Fifty percent of children with stroke will have no identifiable disorder. There is limited published information about the etiology and outcome of childhood stroke and very little evidence in the literature about appropriate management and prevention approaches. Embolic stroke resulting from cardiac disease or carotid dissection and stroke associated with severe prothrombotic conditions (eg, congenital homozygous protein C deficiency or APLA syndrome) appears to benefit from anticoagulation. Warfarin, UFH, or LMWH has been successful in treating and preventing recurrence of acute stroke in children with these underlying disorders. For strokes of other etiologies, anticoagulation does not improve outcome better than treatment with antiplatelet agents. Although the use of thrombolytic agents within 3 hours of initiation of the signs of stroke can be successful in improving outcomes in adults, the safety and efficacy of this strategy in children has not been demonstrated.

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Bleeding disorders

Jorge A. Di Paola and Amy D. Shapiro

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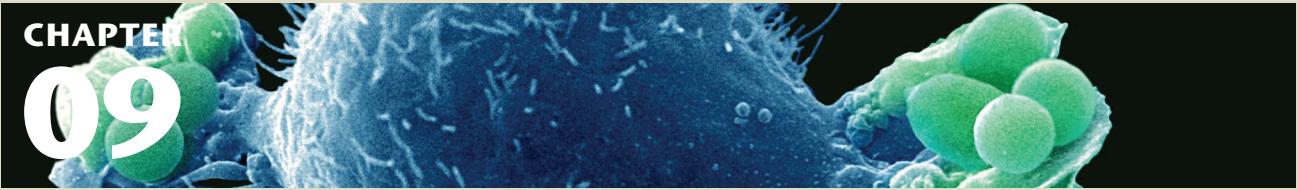
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CHAPTER
09



Bleeding disorders

Jorge A. Di Paola and Amy D. Shapiro

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Overview of hemostasis

Hemostasis is the process through which bleeding is controlled at a site of damaged or disrupted endothelium and is a dynamic interplay between the subendothelium, endothelium, circulating cells, and plasma proteins. Immediately after vessel injury, plasma and cellular components are recruited and activated to reduce bleeding and initiate tissue repair. The hemostatic process often is divided into three phases: the vascular, platelet, and plasma phases. Although it is helpful to divide coagulation into these phases for purposes of understanding, *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 tissue factor and collagen that then come into contact with blood. Platelets bind to von

Willebrand factor (vWF) incorporated into the subendothelial matrix through their expression of glycoprotein Iba (GPIba). 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 glycoprotein (GPVI) and integrin $\alpha_2\beta_1$, resulting in calcium mobilization, activation of the fibrinogen receptor, integrin $\alpha_{IIb}\beta_3$, and subsequent platelet aggregation (Figure 9-1). For a detailed discussion of platelet function, please see Chapter 10.

The *plasma phase* of coagulation is initiated through the exposure of tissue factor (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). The TF:FVIIa:FXa complex converts a small amount of prothrombin to thrombin, resulting in an initial thrombin burst sufficient to cleave factor VIII (FVIII) from vWF and to generate an amplification loop through activation of clotting factors, including FVIII, factor IX (FIX), and factor XI (FXI). These reactions include platelet activation, resulting in the expression of surface platelet factor V (FV) and activation of FV to FVa; activated FIX (FIXa) generated through these noted reactions binds to the surface of activated platelets. Activated FVIII complexed with FIXa forms the potent intrinsic tenase complex, resulting in the conversion of large amounts of FX to FXa, which in association with FVa on the activated platelet surface, results in a thrombin burst sufficient to convert fibrinogen to fibrin (Figure 9-2) and to result in subsequent normal clot formation. The formed clot is stabilized by the thrombin-mediated activation of factor XIII (FXIII) and thrombin-activatable fibrinolysis inhibitor (TAFI). Ultimately, the clot

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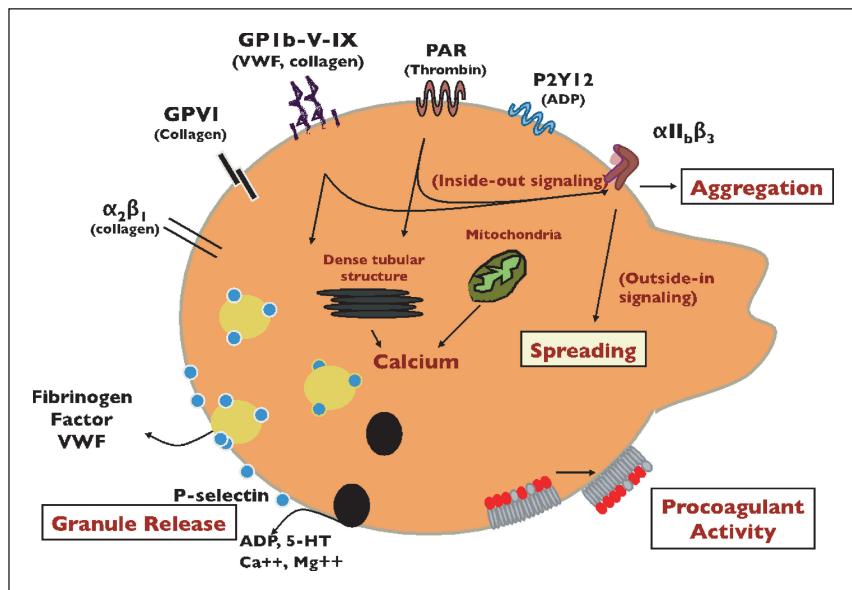


Figure 9-1 Platelet activation. Platelets can undergo activation through stimulation by soluble agonists, such as thrombin, or by contact (adherence) to the subendothelial matrix. This simplified cartoon shows several platelet components, including receptors and granules as well as the pathways of activation and the effect on platelet responses, such as aggregation, spreading, granule release, and procoagulant activity.

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 these processes to the site of injury and quench the reactions to prevent systemic activation and pathologic propagation. The hemostatic system has three main inhibitory pathways mediated through anti-thrombin: the protein C–protein S complex and the tissue factor pathway inhibitor (TFPI). 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:FIIa. Antithrombin released at the margins of endothelial injury binds in a 1:1 complex with thrombin, inactivating thrombin not bound by the developing clot. 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 protein S and inactivates activated FVa and FVIIIa. This negative feedback results in reduced subsequent thrombin generation and quenching of fibrin generation. The fibrinolytic system also includes inhibitors, principally plasminogen inhibitor-1 (PAI-1), which as its name implies, inhibits tPA, and α_2 -antiplasmin (α_2 AP), which inhibits plasmin.

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 will review the approach

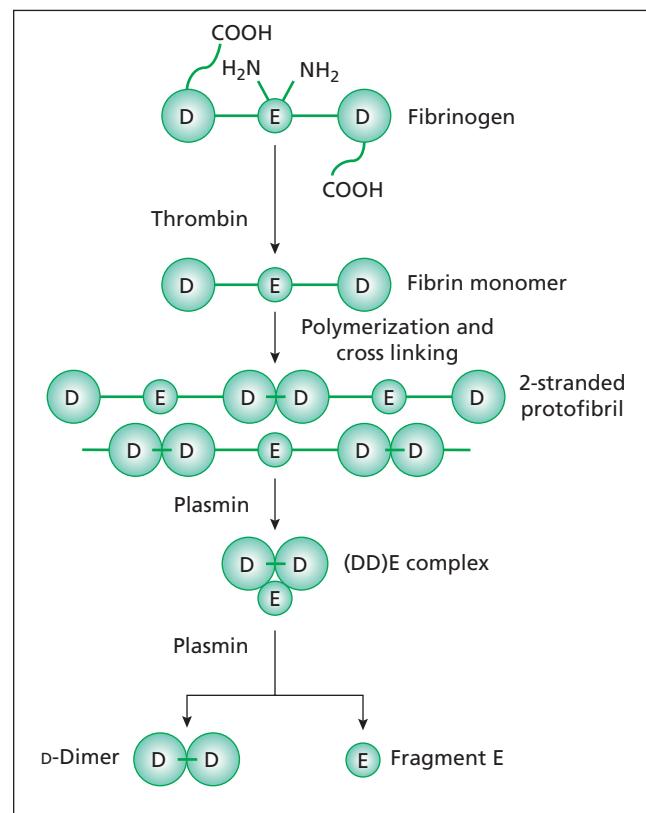


Figure 9-2 Fibrin formation and degradation. Fibrinogen has a trinocular structure with a central E and two D domains. Thrombin cleaves fibrinopeptides A and B from the NH₂ terminal of the A1 and B1 chains, respectively, located in the E domain. The resultant fibrin monomers polymerize nonenzymatically forming protofibrils. Factor XIIIa cross-links the D domains of adjacent 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.

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.
- Defects in primary hemostasis (platelets and vWF) typically result in mucocutaneous bleeding symptoms.
- Defects in coagulation factors cause variable symptomatology but may result in deep tissue bleeding, including intramuscular hematomas, hemarthroses, retroperitoneal, and, occasionally, central nervous system bleeding events.

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 symptom appearance 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, and menorrhagia 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 versus 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 will guide the direction of the laboratory investigation. Quantification of clinical bleeding is a challenge, particularly in the outpatient setting. In recent years, several bleeding assessment tools have been developed to more accurately differentiate bleeding phenotypes in healthy individuals and in patients with bleeding disorders. It is likely that in the future these assessment tools will significantly affect the evaluation of patients. These assessment tools, however, require validation in prospective studies. For now, the personal bleeding history is critical to guide laboratory testing; in addition, a positive family history serves as supportive evidence for a hereditary bleeding disorder, but its absence does not rule this out.

Screening tests

The laboratory evaluation for bleeding includes performance of initial screening tests. 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 to all abnormalities associated with a bleeding disorder, including vWF, mild FIX, FXIII, PAI-1, and α_2 AP deficiencies; therefore, a patient history strongly suggestive of a bleeding disorder may warrant testing for such deficiencies, including rare abnormalities regardless of screening test results. The most common screening tests utilized include the platelet count, prothrombin time (PT), and activated partial thromboplastin time (aPTT). When the PT or aPTT are prolonged, mixing studies are required via a one-to-one 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 or a global inhibitor as best exemplified by a lupus anticoagulant.

Screening tests also are utilized to identify individuals with a high likelihood of von Willebrand disease (vWD) or platelet disorders. The bleeding time, once widely used, has become obsolete because of the lack of sensitivity and specificity. The PFA-100® (platelet function assay) has been proposed to have 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 screening tool, however, has been challenged based on the reported low sensitivity (24%-41%) of the device in individuals with mild platelet secretion defect or storage pool disorders.

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 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. Therefore, the clinical suspicion for a bleeding disorder is critical to determine extent of the laboratory investigation.

Key points

- Patients with bleeding disorders occasionally may 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.

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 by 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 10. 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 9-1. Platelet activation is the result of multiple signaling pathways that culminate into the 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 GPIba-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 bipolar 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 function

defects is included in Chapter 10, 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 GPIba 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 δ -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, a severe platelet function defect. Most platelet function defects are diagnosed via standard assays. 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 the result of medical interventions such as cardiopulmonary bypass. The list of medications associated with platelet dysfunction is vast. The most commonly used medications that result in platelet dysfunction, many of which are over the counter, include aspirin and other nonsteroidal anti-inflammatory drugs, antihistamines, guaifenesin, certain anticonvulsants (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 gingko biloba, also have been reported to affect platelet function. Thus, when obtaining a medical history, it is imperative to ask not only about prescribed medications but also over-the-counter and herbal supplements. Most of these medications and supplements will 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 interpret laboratory tests.

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 defects 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 10. Briefly, the platelet count must be determined and the smear reviewed; platelet aggregation assays will be abnormal in the setting of significant thrombocytopenia (ie, $<100 \times 10^9$), and the PFA-100[®] will be abnormal with significant thrombocytopenia or anemia. Thus, a complete blood count (CBC) should be performed before obtaining platelet-specific studies. The two commonly available tests to screen for platelet function disorders both have limitations. The original screening test was the bleeding time; as previously stated, the 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 and commonly in types 2 and 3 vWD. The usefulness of the PFA-100[®] CT in the diagnosis of type 1 vWD is controversial (see discussion in the section on vWD). A significant limitation of the PFA-100[®] is the fact that the CT is affected by the platelet count and hemoglobin levels. The CT will be abnormal if the platelet count is less than 100,000/ μ L and the hemoglobin is <10 g/dL. Patients with severe platelet function defects, such as Bernard-Soulier and Glanzmann thrombasthenia, also will have abnormal results. The CT is often abnormal in patients on aspirin, clopidogrel, and ticlopidine. 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 two most common bleeding disorders (ie, platelet function defects and vWD). These aspects significantly limit its use as a screening test. 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 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. 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. Luminometry, commonly used in combination with platelet aggregation, provides a sensitive evaluation of ATP release from dense granules. ATP 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. Platelet aggregation testing is labor intensive and expensive.

As with the PFA-100® CT, many medications and supplements have been reported to affect platelet aggregation studies; therefore, if possible, the assay should be performed when patients are no longer receiving these medications or supplements for approximately 10 days. This assay can be performed in anemic and even thrombocytopenic patients (if one suspects a platelet function defect in addition to thrombocytopenia) as 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 reliable reported results.

Flow cytometry

Flow cytometry may be employed to quantify levels of platelet surface receptors and can confirm the diagnosis of Bernard-Soulier and Glanzmann thrombasthenia. In some institutions, these assays are available and have become the method of choice for diagnosis.

Some platelet function defects lead to easily identifiable platelet ultrastructural changes visualized by electron microscopy. In particular, patients with a deficiency or absence of dense bodies (δ -storage pool deficiency) or α -granules (gray platelet 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.

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 will resolve 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.

Several medications enhance hemostasis nonspecifically and are useful in the face of platelet dysfunction. These include desmopressin, antifibrinolytic agents, estrogen, and recombinant FVIIa (rFVIIa). Desmopressin may improve platelet function in many congenital disorders, uremia, and during cardiopulmonary bypass; the specific mechanism of action is not clear. Desmopressin may be administered intravenously, subcutaneously, and, for home management, intranasally. Intranasal use requires the highly concentrated solution (Stimate®; CSL Behring, King of Prussia, PA) as the intranasal formulation commonly used to manage diabetes insipidus or enuresis is ineffective as a hemostatic agent. 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 between the results of platelet function tests and clinical outcomes exists, and thus, the value of this approach is uncertain. Desmopressin is a safe agent, although its use can lead to vasodilation, resulting in 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 and hyponatremia. Although this rarely occurs in adults and older children, the risks are increased in young children and in those receiving intravenous fluids. Therefore, an experienced care provider should oversee its 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 tranexamic acid [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. These agents 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 menorrhagia. 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 as 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 menorrhagia with both the previously mentioned hemostatic effects and with 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 menorrhagia 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 risks associated with estrogens include thrombosis; thus, these agents should be avoided in patients with a 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. This agent is licensed in the European Union for the management of bleeding in patients with Glanzmann thrombasthenia refractory to platelet transfusions. 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. Although off-label, the use of rFVIIa in patients with severe bleeding in whom standard therapeutic measures have failed is a reasonable guideline adopted by many institutions. For severe bleeding unresponsive to the previously mentioned measures, especially in patients with Bernard-Soulier 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 risk of platelet transfusions). A more important specific risk associated with Bernard-Soulier 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.

The benefits of local measures in the management of bleeding episodes for which these approaches are applicable should be emphasized. Application of direct pressure is an effective measure for epistaxis, oral bleeding, and cutaneous bleeding. For accessible bleeding sites, including the nose, mouth, and skin, the use of topical adjunctive agents are also effective and safer than systemic therapy.

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 (HLA)-compatible sibling is available. Patients with platelet function disorders should receive thorough education regarding their condition and its management so that bleeding episodes are recognized early, managed at home, or prevented through appropriate measures or interventions. Important, patients should be advised to report their condition to physicians before undergoing invasive procedures so that appropriate prophylactic measures are used to prevent bleeding; in addition, all new medications should be checked for their ability to interfere with platelet function.

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 complexity of platelet structure and function makes 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 test 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 menorrhagia.

von Willebrand disease

Pathophysiology

vWD is the most common bleeding disorder with a reported prevalence of symptomatic disease that ranges from 1/100 to 1/10,000. The transmission of vWD is autosomal dominant for most types but also can be rarely inherited in a recessive manner.

vWD is caused by the quantitative or qualitative deficiency of vWF, which is a large, multimeric glycoprotein produced in megakaryocytes and endothelial cells. Therefore, two pools of vWF are available for normal hemostasis. Plasma vWF, mostly released from stored vWF in Weibel-Palade bodies in endothelial cells, and platelet vWF that is stored in α -granules and released upon platelet activation. The main roles of vWF in hemostasis are to promote platelet adhesion to the exposed subendothelium and to serve as a chaperone for factor VIII (FVIII) in plasma, protecting it from proteolytic degradation. vWF undergoes dimerization in the

endoplasmic reticulum (ER), glycosylation in the ER and Golgi complex, and multimerization in the Golgi complex and is packed into storage granules after cleavage of the vWF propeptide (vWFpp). The vWFpp is released in equimolar concentrations to the mature vWF molecule. The vWFpp therefore is useful to measure the rate of clearance of mature vWF. When multimers are secreted into the blood, the largest (also called ultralarge) vWF multimers are cleaved by the metalloprotease adisintegrin and metalloprotease with thrombospondin (ADAMTS13). Recent data suggest that vWF clearance is led in part by macrophages in the liver and spleen.

Classification of vWD

vWD often is categorized into quantitative or qualitative vWF defects. Although vWD type 1 and type 3 represent partial and absolute quantitative defects respectively, vWD type 2 is characterized by a qualitative vWF defect. Following is a brief description of the different subtypes and the molecular mechanisms that define them. Figures 9-3 and 9-4 illustrate these mechanisms and how they lead to the current classification. Table 9-1 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 others with clinical symptoms is usually present, yet their absence does not preclude the diagnosis. Those patients with vWF levels <20 IU/dL usually have identifiable mutations in the vWF gene (*vWF*) and commonly are associated with significant bleeding symptoms. 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 two 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 type 1C vWD have a robust initial response to desmopressin, but they exhibit an abrupt vWF level decrease within 2-4 hours.

A consistent diagnostic criterion is difficult to achieve as not all individuals that inherit a mutation in vWF show signs

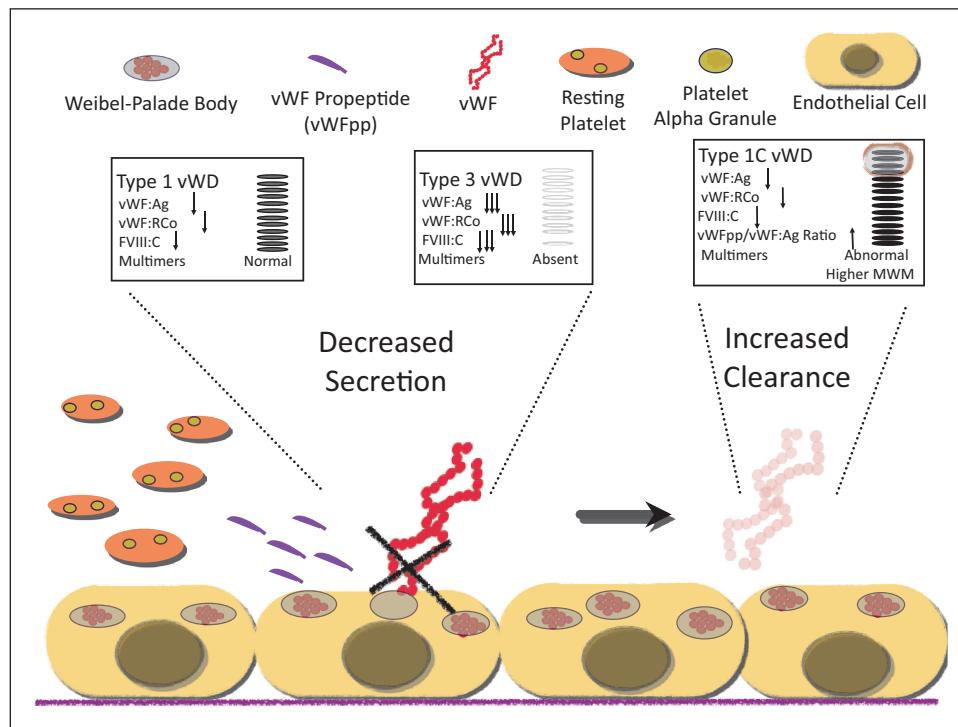


Figure 9-3 Mechanisms of disease for vWD types 1 and 3. Note that in boxes are shown the most common laboratory findings for these types. From Hematology 2012, the ASH Education Program.

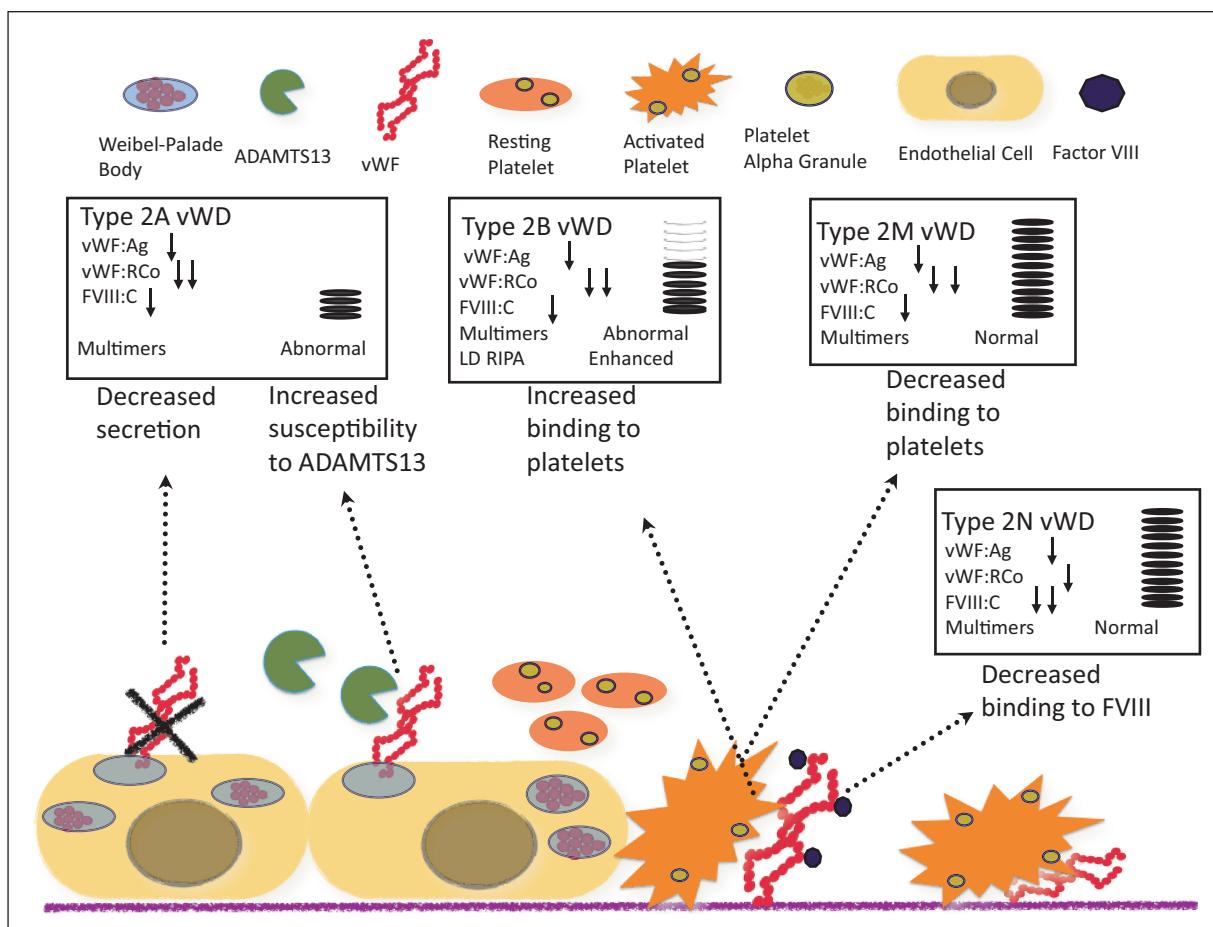


Figure 9-4 Mechanisms of disease for vWD types 2. Note that in boxes are shown the most common laboratory findings for the different subtypes. From Hematology 2012, the ASH Education Program.

Table 9-1 Classification and diagnosis of von Willebrand disease.

Condition	Description	vWF:RCO (IU/dL)	vWF:Ag (IU/dL)	FVIII	vWF:RCO/ vWF:Ag
Type 1	Partial quantitative vWF deficiency (75% of symptomatic vWD patients)	<30*	<30*	↓ or Normal	<0.5-0.7
Type 2A	Decreased vWF-dependent platelet adhesion with selective deficiency of high-molecular weight multimers	<30*	<30-200* †	↓ or Normal	<0.5-0.7
Type 2B	Increased affinity for platelet GPIb	<30*	<30-200* †	↓ or Normal	Usually <0.5-0.7
Type 2M	vWF-dependent platelet adhesion without selective deficiency of high-molecular weight ↓ multimers	<30*	<30-200* †	or Normal ↓	<0.5-0.7
Type 2N	Markedly decreased binding affinity for FVIII	30-200	30-200	↓↓	<0.5-0.7
Type 3	Virtually complete deficiency of vWF (severe, rare)	<3	<3	↓↓↓ (<10 IU/dL)	Not applicable
Low vWF		30-50	30-50	Normal	<0.5-0.7
Normal		50-200	50-200	Normal	<0.5-0.7

↓ 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; some patients with type 1 or type 2 vWD have levels of vWF:RCO or vWF:Ag of 30-50 IU/dL.

† The vWF:Ag in the majority of individuals with type 2A, 2B, or 2M vWD is <50 IU/dL.

** This does not preclude the diagnosis of vWD in patients with vWF:RCO of 30-50 IU/dL if there is supporting clinical or family evidence for vWD, nor does this preclude the use of agents to increase vWF levels in those who have vWF:RCO of 30-50 IU/dL and who may be at risk for bleeding.

of clinical disease (phenomenon known as low penetrance) and not all individuals that inherit the same mutation show the same clinical signs (known as variable expressivity). More than 50% of individuals with vWF levels in the mildly decreased range (30-50 IU/dL) are asymptomatic or have minimal bleeding symptoms. Therefore, the presence of plasma vWF levels between 30 and 50 IU/dL does not automatically define vWD type 1. Individuals with blood group O have 25%-30% lower vWF levels as compared with those who have blood group A; therefore, 14% of blood group O individuals in the United States are expected to have vWF levels equal to or lower than 50 IU/dL. On the basis of the fact that mild bleeding commonly is reported in the healthy population, it is possible that many individuals diagnosed with mild vWD type 1 may not have genetically inherited vWD but rather an association of mild decreased vWF levels (within the established range for blood group O) and mild bleeding symptoms. Regardless of causality, most individuals in this category likely benefit from similar therapeutic measures used for patients with vWD type 1.

vWD type 2

vWD type 2 is subclassified into type 2A (loss of intermediate- and high molecular-weight multimers because of decreased secretion or increased susceptibility to ADAMTS 13), type 2B (gain-of-function mutation resulting

in spontaneous vWF-platelet binding under physiologic shear conditions, resulting in 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 decreased 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 very low FVIII:C levels (<5%), representing the steady state of factor VIII 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 and genitourinary tracts. 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.

Menorrhagia, 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 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 menorrhagia resulting in early hysterectomy has been observed in females with a variety of subtypes including types 1, 2, and 3. Because bleeding manifestations of vWD include commonly observed symptoms in the normal population, such as bruising, epistaxis, and menorrhagia, clinical suspicion is important for timely and accurate diagnosis.

Diagnosis of vWD

Screening tests

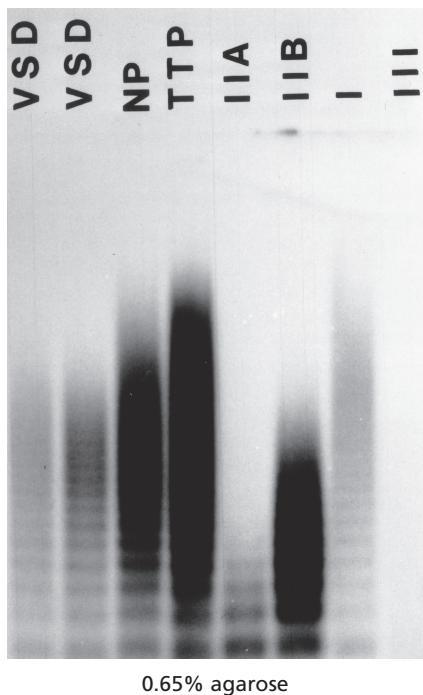
Screening tests have several limitations in predicting the diagnosis of a bleeding disorder. These limitations are especially relevant to vWD. For example, the aPTT, which has been proposed as a screening test for vWD, is often normal in most cases of types 1 and 2 vWD. The aPTT yields abnormal results only if the FVIII is reduced sufficiently to be detected by the assay. Therefore, the aPTT is only noticeably abnormal in patients with types 2N and 3 vWD, and sometimes in type 2B. The PFA-100® CT also has been proposed as a screening test for individuals with suspected vWD; as previously discussed, this test is sensitive only to very low vWF levels and therefore patients with vWD often are missed. In summary, screening laboratory methods 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 tests

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 activity and binding to vWF (FVIII activity and FVIII-binding assay). Also the vWF multimers distribution is used to differentiate subtypes. A potential role

for assays that measure the binding of vWF to collagen has been described. On the basis of these results, further testing, described in further detail later, may be pursued. Limitations exist with several of these assays. Both vWF and FVIII are acute-phase reactants and may increase two to five times above baseline because of a variety of conditions or circumstances, including but not limited to elevated estrogen levels, as with oral contraceptive agents or due to 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. It is common for patients who have undergone serial testing to have moderate variations in levels over time. Because of the difficulty in ruling out this disorder with one 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.

vWF:RCo is widely used and is accepted as the gold standard for vWF activity. The assay can be difficult to interpret as it exhibits a high coefficient of variability when the vWF:RCo is lower than 15 IU/d, and it may affect differentiation between type 1 and type 2 vWD. Latex immunoassay and ristocetin enzyme-linked immunoadsorbent assay (ELISA) currently are under evaluation for their clinical utility as alternatives for the traditional vWF:RCo assay. A recent report suggests that the vWF:RCo assay can be abnormal in a subset of otherwise-healthy African Americans due to the presence of a common single nucleotide polymorphism (SNP) in exon 28 of vWF. This SNP appears to affect ristocetin binding without conferring an evident hemorrhagic risk and potentially may lead to a false diagnosis of vWD type 2. Newly developed functional assays utilizing the platelet ligand for vWF, GPIb, may allow for assessment of vWF activity without the need for ristocetin, thus improving discrimination of type 2 variants without the noted pitfalls. Low-dose ristocetin-induced platelet aggregation (LD-RIPA) is used to identify abnormally increased binding of vWF to platelets. 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 vWD type 2, and their absence easily identifies vWD type 3 (Figure 9-5). The FVIII activity level and FVIII-binding assay



0.65% agarose

Figure 9-5 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 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 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 AWS with the loss of multimers of all sizes, and thrombotic thrombocytopenic purpura in which ultralarge multimers can be observed.

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. 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 greater than 2 standard deviations below the population mean and the plasma vWF multimer distribution is normal. Additionally the vWF:RCo/vWF:Ag ratio approximates 1. In patients with vWD type 1C, the vWF:Ag and vWF:RCo are low and 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.

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 high-molecular weight multimers (HMWM). The vWF:RCo/vWF:Ag ratio approximates 0.5. Type 2M is caused by mutations in the platelet glycoprotein 1b α (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 two entities as treatment approaches are significantly different. vWD type 2B is treated with vWF concentrates as the molecular defect is in vWF, whereas pseudo-vWD is treated with platelet transfusions as it is caused by mutations in platelet GPIb. If pseudo-vWD is suspected, the patient's platelets are tested with a normal exogenous vWF substrate for evaluation 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 two proteins. Patients with vWD type 2N may exhibit normal or decreased vWF:Ag and vWF:RCo with disproportionately decreased FVIII:C. Patients with this diagnosis may be misclassified as mild factor deficiency. 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 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 <5%, and lack of multimers. A description of the laboratory pattern for each subtype is shown in Table 9-1

Genetic testing

Sequencing of vWF gene is challenging due to its large-size, highly polymorphic structure, and presence of a homologous partial pseudogene in chromosome 22. Therefore, gene sequencing for diagnosis currently is reserved for specific cases in which these test results will likely contribute signifi-

cantly to diagnosis and management. Genetic testing may be justified in vWD type 3 as large deletions may predispose to the development of inhibitory antibodies and anaphylactic reactions. Also, gene sequencing could be useful in cases in which treatment options vary based on diagnosis, such as in vWD type 2N.

Acquired von Willebrand syndrome

Acquired von Willebrand syndrome (AWS) is a rare disorder in which vWF is synthesized normally but cleared from the circulation more rapidly. Several conditions have been associated with AWS. Three mechanisms are associated with the observed increased clearance: (i) autoantibodies against vWF and immune complex formation (eg, hypothyroidism due to Hashimoto's thyroiditis), (ii) vWF binding to cancer cells (eg, Wilms tumor, lymphoproliferative disorders), and (iii) increased proteolytic activity of HMWM under pathological high-shear stress conditions (eg, congenital heart disease, aortic stenosis, angiodysplasia). The laboratory diagnosis and management of AWS does not differ significantly from the congenital forms. Treatment of the underlying disorder leading to AWS often resolves the defect.

Treatment

The principles of management of vWD are 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 vWF-containing concentrates derived from human plasma. Mild to moderate bleeding associated with type 1 vWD often is managed with desmopressin, most commonly with the intranasal preparation, and antifibrinolytic agents as required. 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 section, should be performed to document a hemostatic response; in vWD, the vWF:Ag, vWF:RCo, and FVIII levels are performed before and 60–90 minutes after the dose, depending on the route of administration. Repeat laboratory evaluation at 4 hours post dose may be appropriate when an altered half-life of the native protein is suspected, as observed in type 1C. Approximately 90% of patients with type 1 vWD 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. Thus, use of desmopressin no more than once daily and no more than on 2–3 consecutive days serves as an acceptable

clinical guideline for home use. There are some reports of the benefits of desmopressin in type 2 vWD; 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. For these reasons, patients with type 2 vWD most commonly are treated with exogenous normal vWF replacement via a concentrate. 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 other 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 U.S. Food and Drug Administration (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. As with all human plasma products, a potential for allergic reactions also exists; however, these are infrequently reported with these products. Administration of the first dose in a hospital or clinic setting may be considered.

Antifibrinolytic agents are useful adjunctive therapies and are used in a similar fashion as described for platelet defects. Conjugated estrogens and oral contraceptive agents are effective therapies for the management of menorrhagia. 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 Platelet function disorders. 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.

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; recently published treatment guidelines published by the National Heart, Lung, and Blood Institute 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 menorrhagia, and vWF concentrates for severe bleeding or in types 2 and 3.

Disorders of secondary hemostasis

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 lead to a clot with poor structural integrity visualized by electron microscopy; formation of large, coarse fibrin strands as opposed to normal thinner strands that form a tight network are observed. In addition, reduced thrombin generation results in decreased generation of activated FXIII 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 normal 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. These deficiencies commonly are observed in males due to their hemizygous state.

Heterozygous females may have factor levels observed in the mild hemophilia range as a result of nonrandom X chromosome inactivation. These women may be more appropriately classified as having mild hemophilia and treated accordingly for bleeding episodes. Rarely, females may have levels in the severe or moderate deficient range because of skewed lyonization 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 determine the severity of disease. In approximately 25% of cases, no family history is identified. In such cases, either the affected individual has a de novo mutation arising in either the patient's or—in the case of the intron 22 inversion (the most common mutation causing hemophilia A)—the maternal grandfather's germ cells during meiosis likely due to single unpaired X chromosome. F8 intron 22 inversions account for ~45% of severe hemophilia A cases.

Rarely, hemophilia can be acquired as a result of the development of autoantibodies most commonly directed against FVIII. This condition, also known as acquired hemophilia, has been associated with a variety of conditions, including pregnancy, malignancies, and advanced age. In ~50% of cases, no known associated disorder can be identified. These 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 small, whereas soft tissue, abdominal, and retroperitoneal hemorrhage are more frequent.

Clinical presentation

The clinical presentation of congenital hemophilia is highly variable and is correlated with the level of deficiency. In infants born to known female carriers, the diagnosis most often can be established at birth by assaying FVIII or FIX from umbilical cord blood. Prenatal testing is available if the genetic defect has been identified within the family; this testing may not be required when the knowledge gained would not alter the course of pregnancy or the planned mode of delivery. 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 or 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%-3%. 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 a procedure. Bleeding symptoms include deep tissue, muscle, or joint bleeding; mucocutaneous bleeding is a common presentation due to increased 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. For patients without a documented family history, the age of presentation is highly variable; excessive bleeding always is associated with injury or surgery. Patients with mild hemophilia typically present later in childhood or during the teenage or adult years.

Joint disease, or hemophilic arthropathy, remains a major morbidity. Although preventive therapy is effective (see Treatment section for details), patients occasionally may present with recurrent hemarthroses, ultimately leading to joint disease. It is not uncommon for patients who have not received optimal treatment, such as those who emigrated from developing nations, to present with hemophilic arthropathy.

Acquired hemophilia may present with the dramatic onset of either mucocutaneous or internal bleeding. Hemarthroses are uncommon. Life-threatening bleeding with associated significant morbidity and mortality are observed.

Diagnosis

The laboratory diagnosis of hemophilia begins with screening coagulation studies, including the PT and aPTT; the aPTT is almost always abnormal. It is important to be cognizant of circumstances in which the aPTT may be normal, especially in mild deficiencies (Figure 9-6). After identification of a prolonged aPTT, a mixing study with normal plasma is performed. Correction of the prolongation points to a factor deficiency, and therefore, specific factor analyses are performed, including FVIII and FIX. The type and level of severity of hemophilia are thereby established. As previously discussed, appropriate specimen procurement and handling are critical to obtain accurate results. In newborns 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

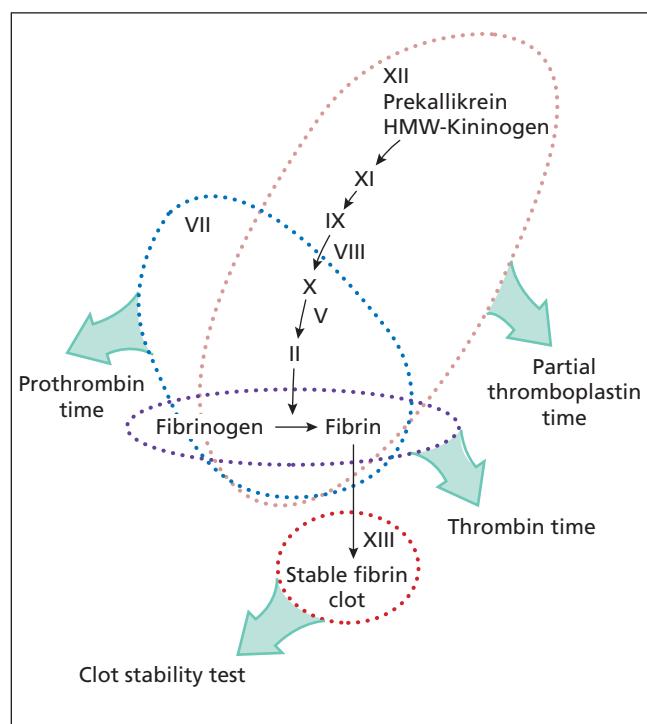


Figure 9-6 Plasma coagulation reactions in in vitro laboratory assays. Factor XII, prekallikrein, and high-molecular-weight kininogen are required for a normal partial thromboplastin time but not for normal in vivo hemostasis. Also, plasma factor XI may not always be required for normal in vivo hemostasis. Platelets and tissue factor are required for normal in vivo hemostasis but are supplied by exogenous reagents in the laboratory assays. This diagram outlines the coagulation factors required for each of four basic tests.

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 based on their clinical circumstances. In addition, mild FIX and FVIII deficiency may be associated with a normal aPTT in some laboratories or circumstances; therefore, if a clinical suspicion for hemophilia exists, FIX and FVIII activity levels should be obtained in addition to the normal screening test.

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 9-2 and 9-3). The choice of the specific product used includes consideration, among other things, of availability, cost, and method of manufacture. Both recombinant and plasma-derived products are

Table 9-2 Factor VIII concentrates currently available in the United States.

Brand name	Generation	Pd versus R	Presence of human proteins	Stability at RT
Monoclate	NA	Pd	Albumin	No
Hemophil M	NA	Pd	Albumin	No
Recombinate	1st	R	Albumin	No
Kogenate FS	2nd	R	Albumin in processing, not final product	Yes: 3 months
Helixate FS	2nd	R	Albumin in processing, not final product	Yes: 3 months
Advate	3rd	R	None	Yes: 6 months
Xyntha (B-domain deleted)	3rd	R	None	Yes: 6 months

Other FVIII concentrates approved for use in FVIII deficiency; these may also contain vWF and are not in general use. NA = not applicable; RT = room temperature; PD = plasma derived; R = recombinant.

available, and decisions of product used should be made in consultation with the patient and family. Typically, 1 IU/kg of FVIII will increase the FVIII level by 2%; doses can be repeated as needed approximately every 8-12 hours. With FIX, dosing depends on the product used—plasma-derived FIX (pdFIX) or recombinant FIX (rFIX). With pdFIX, 1 IU/kg increases the FIX level by 1%, whereas with rFIX, the level increases by 0.6%-0.8%, with children exhibiting a lower recovery compared with adults. FIX doses can be repeated every 12-24 hours as needed.

Treatment approaches are divided into two main categories: prophylaxis and on demand. Prophylaxis is the regular infusion of factor replacement to prevent or suppress bleeding events. Primary prophylaxis is the initiation of replacement therapy before or shortly after the first hemarthrosis and has been proven to be the most effective approach to prevent the development of joint disease. Therefore, primary prophylaxis should be considered the optimal therapy for severe hemophilia; however, when it should be instituted and when or if it should be stopped remain controversial. In Sweden, where prophylaxis was pioneered, therapy is initiated before the first joint bleed commonly between 9 and 12 months of age. In the United States, a common approach is to wait until 1-2 hemarthroses have occurred because some patients even with severe hemophilia may not experience a hemarthrosis until several years of age, thereby limiting invasive therapy until required. Prophylaxis is time and resource intensive and requires adequate venous access often necessitating a central venous catheter; therefore, there may be a

benefit to institute therapy after hemarthrosis has occurred to demonstrate its necessity. The negative effect of this approach is that even one significant hemarthrosis may result in joint damage; in addition, this approach allows sub-clinical bleeding, a potential although as yet not well-defined contributor to joint disease. Once primary prophylaxis has been instituted, it should be continued throughout childhood. The topic of continued prophylaxis into adulthood is an ongoing area of research.

Secondary prophylaxis is the regular infusion of replacement therapy as described earlier but after the onset of significant hemarthroses or joint disease to interrupt a bleeding pattern or prevent further joint damage through suppression of bleeding episodes. Joints with repeated bleeding develop acute or chronic synovitis, followed by articular damage; the process of repeated bleeding in a joint is termed target joint. The bleeding pattern in target joints has been documented to be amenable to secondary prophylaxis. Prophylaxis may be administered for specific patients in circumstances that require adequate hemostatic coverage, such as before sports. 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 deficient hemophilia require this therapy because of their bleeding pattern. Secondary prophylaxis and limited prophylaxis are used in all severities of hemophilia based on circumstances that warrant adequate hemostatic coverage. Issues related to prophylaxis include adherence, cost, and the need for adequate venous access; prophylaxis and the associated issues have been reviewed. Several prophylactic and general treatment-dosing approaches exist and are detailed in Table 9-4. Although prophylaxis is effective in the prevention of the majority of spontaneous bleeding events, patients who experience breakthrough bleeding episodes require immediate treatment according to the recommendations in Table 9-4.

Table 9-3 FIX concentrates currently available in the United States.

Brand	Pd versus R	Presence of human proteins		Stability at RT
Mononine	Pd	FIX and others		No
Alphanine	Pd	FIX and others		No
Benefix	R	w		Yes (for 6 months)

Pd = plasma derived; R = recombinant.

Table 9-4 Typical dosing for FVIII and FIX deficiency in different clinical circumstances.*

Factor	Joint/muscle	Life or limb threatening	Preoperative	Prophylaxis
FVIII	25 IU/kg Repeat as needed	50 IU/kg Multiple doses required	50 IU/kg	25-40 IU/kg three times weekly or every other day
pdFIX	50 IU/kg Repeat as needed	100 IU/kg Multiple doses required	100 IU/kg	50 IU/kg twice weekly
rFIX	60-70 IU/kg Repeat as needed	120-140 IU/kg Multiple doses required	120-140 IU/kg	60-70 IU/kg twice weekly

*Represent general dose guidelines, practice varies. The volume of distribution of infused rFIX is ~1.2 in adults, and ~1.4 in children. Therapy duration for intracranial hemorrhage varies but is minimally ~2 weeks; in children intracranial hemorrhage should prompt consideration for ongoing prophylactic therapy. Prophylaxis regimens vary; listed doses typically utilized in Swedish regimens.

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 and is less expensive in the short run, but is ineffective in the prevention of joint disease. This mode of therapy now is used primarily for patients with moderate and mild deficient hemophilia due to the infrequency of bleeding events and the associated low risk of joint disease. On-demand therapy may be used by adults with severe disease who experience fatigue with the requirements of prophylaxis or who feel it is not required. The typical initial dosing for bleeding episodes can be found in Table 9-4. Infusion therapy for hemophilia, regardless of the regimen used, is best delivered in the home setting to allow for prophylaxis or prompt therapy. Family members and patients are trained to administer the factor concentrate at home without the need for a medical facility.

Adjunctive therapy for hemophilia is similar to that discussed for platelet defects and vWD. Patients with mild FVIII deficiency 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 concentrate or desmopressin. For women with hemophilia with menorrhagia, hormonal suppressive therapy 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 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 and mortality. The incidence of inhibitors is between 20% and 35% in severe, previously untreated, FVIII-deficient patients and <5% in severe FIX-deficient patients. The present inhibitor prevalence is approximately 10% in FVIII

deficiency and 3%-5% in FIX deficiency. Risk factors for inhibitor development include both patient- and environmental-related issues. 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 gene, such as large deletions, are associated with increased risk. In addition, patients of African or Hispanic ethnicity have a significantly higher rate of inhibitor development. Environmentally related risk factors have been purported to include the source of the factor product used (plasma derived vs. recombinant); these data remain controversial. A recent systematic review suggested that the rate of inhibitor formation in severe FVIII deficiency is twofold higher in patients who received recombinant FVIII versus those who received plasma-derived FVIII. A prospective study is under way to confirm or refute this finding.

Inhibitors are divided into two 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) despite repeated exposure or stimulation, whereas high-responding inhibitors are those that achieve a titer >5 BU at any time regardless of present titer. Patients with high-responding inhibitors may exhibit a decrease in or an undetectable 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 will demonstrate an increase in inhibitor titer in 7-10 days after exposure. Stimulation and increase of inhibitor titer is termed 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.

Patients with low-responding inhibitors commonly are managed with higher doses of standard replacement therapy

calculated to overcome the inhibitor titer and achieve a hemostatic level. A minority of patients have low-titer inhibitors that resolve without intervention (often within a few weeks) and are termed transient inhibitors; therefore, ongoing measurement of titers is important to document persistence and for dose calculation. Patients with high-responding inhibitors are not able to achieve a hemostatic level with standard replacement therapy and thus are treated with alternative hemostatic products termed bypassing agents.

The three 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. Inhibitor eradication, also called immune tolerance induction (ITI), requires regular administration of the deficient factor to reset/tolerize the immune system. An international prospective ITI study in good-risk patients recently was completed and published (Hay et al., 2012). This study compared daily high-dose FVIII (200 IU/kg/d) to lower dose FVIII (50 IU/kg/d) three times weekly. The study was stopped before reaching the planned endpoint because of an increased rate of bleeding observed in patients on the low-dose arm. Typical ITI regimens may include either of these infusion schedules or a regimen of 100 IU/kg given once daily. Retrospective registries have identified several factors affecting ITI success, including the peak inhibitor titer, titer at start of therapy (<10 BU associated with improved outcome), age at initiation, and time from inhibitor development to ITI start. It is best to initiate ITI when the titer is <10 BU, although this must be balanced against the risk of delaying tolerance. Inhibitor development in FIX deficiency 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. For such patients, 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 represent a significant treatment challenge for practitioners.

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. Two bypassing agents are available for the management of bleeding in inhibitor patients, activated

prothrombin complex concentrate (APCC; FEIBA, Baxter, Westlake Village, CA) and rFVIIa (NovoSeven, Novo Nordisk, Bagsvaerd, 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 mechanism of action of rFVIIa is through thrombin generation on the surface of activated platelets through tissue factor-dependent and –independent mechanisms. 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. Another consideration is that rFVIIa does not stimulate either the FVIII or FIX inhibitor titer and may be preferred if trying to allow the inhibitor to reach a low level before ITI initiation. APCC may contain small quantities of FVIII and result in continued stimulation of the inhibitor titer in FVIII-deficient patients. 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 9-5). 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. Although the approach has 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 was confined to prevention of bleeding during invasive procedures. Because of obvious concerns for hemostatic control during surgery and postoperatively with bypassing agents and concern for thrombotic events with repeated use in a high-risk setting, inhibitor patients were not offered elective surgery until fairly recently. Few studies demonstrating successful hemostatic strategies for inhibitor patients in

Table 9-5 Typical dosing for currently available bypassing agents.

Agent	Joint/muscle	Life or limb threatening	Preoperative	Prophylactic
APCC*	50-75 U/kg Repeat every 8-12 hours as needed	75-100 U/kg Repeat every 12 hours	50-75 U/kg	75 U/kg three times weekly
rFVIIa	90-120 mcg/kg	90-120 mcg/kg	90-120 mcg/kg	90 mcg/kg/day
Standard dose†	Repeat every 2-3 hours as needed	Repeat every 2-3 hours		
rFVIIa	270 mcg/kg	270 mcg/kg	No data	270 mcg/kg/day
High dose	Data not available on follow-up doses required	Data not available on follow-up doses required		

*Doses >200 U/kg/day contraindicated per prescribing information. APCC is licensed for the treatment of bleeding, not for surgery or prophylaxis.

†The licensed dose of rFVIIa in the United States is 90-120 mcg/kg for treatment and prevention of bleeding during surgery; not approved for prophylaxis.

the surgical setting have been performed. Over the past decade, several prospective studies have demonstrated the successful use of rFVIIa for both minor and major surgery (see Table 9-5 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.

Recently, prophylaxis with bypassing agents to prevent bleeding episodes in inhibitor patients has gained attention as a potentially feasible approach. Several case reports of rFVIIa used prophylactically led to the performance of a prospective study that demonstrated an approximately 50% reduction in bleeding episodes during prophylaxis in patients with a high frequency of bleeding. A number of case series have demonstrated the use of APCC for prophylaxis with mixed results; a prospective study is under way. Currently, several new agents are in development with potential improved hemostatic properties and longer half-lives that may improve the overall treatment of inhibitor patients and make prophylaxis more effective and feasible in the future.

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. Inhibitor eradication in acquired hemophilia is different than in congenital hemophilia complicated by inhibitors. Because acquired hemophilia is due to the development of autoantibodies that result from loss of self-tolerance, they tend to respond to immunosuppressive medications effective in autoimmune

disorders in general. Although these patients are too few to allow for well-designed prospective studies, a number of reports have demonstrated the effectiveness of glucocorticoids, cyclophosphamide, and more recently rituximab, with order of use as listed respectively. Although ITI has been reported in acquired hemophilia, it is more cumbersome than immunosuppression alone and usually is not required.

New therapies

Recently, new approaches to prolong the half-life of exogenously administered coagulation concentrates have been reported. Different strategies have been employed, including approaches to alter clearance, such as sialic acid residues addition or hydrophilic polymer conjugation, including coagulation factors encapsulated in polyethylene glycol (PEGylated) liposomes. Additionally, fusion proteins rendered resistant to normal clearance pathways are being explored. The use of products that increase the half-life of exogenous clotting factors has the potential to decrease dosing frequency in prophylactic regimens, positively improving compliance and quality of life, and also may improve on-demand therapy options through a decreased requirement for repeated dosing. These products are being explored actively in clinical trials. Finally, a major breakthrough in the field of hemophilia was recently reported. Nathwani et al. (2011) reported in the *New England Journal of Medicine* the result of a trial in which six patients with hemophilia B were treated with one infusion of an adenovirus-associated virus (AAV) vector expressing FIX. All patients showed expression of coagulation FIX for >6 months and their exogenous factor infusions were reduced significantly.

Prognosis and outcomes

Currently, patients with severe hemophilia without inhibitors treated on a prophylactic regimen have an excellent

prognosis and lead near-normal lives commonly without the development of hemophilic arthropathy. 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 will have experienced hemarthroses, muscle, or even intracranial hemorrhage and that some of these bleeding events will be 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. Development of improved therapeutic approaches for inhibitor patients who still experience increased morbidity and mortality compared with noninhibitor patients are required. 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 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 success has been reported. The FIX-deficient inhibitor population with anaphylactoid reactions represents a small vulnerable population with only one 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, and severe depending on the factor level.
- Patients with severe hemophilia are at risk for the development of joint disease termed hemophilic arthropathy that can be prevented by regular factor infusions begun at an early age (prophylaxis).
- 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 or replaced factor-termed inhibitors; inhibitors are divided into high- and low-responding types, and the presence of an inhibitor may render standard substitutive therapy ineffective.
- Inhibitors can be eradicated through treatment regimens termed ITI.
- Patients with high-responding inhibitors are treated with bypassing agents to manage their 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.

Rare factor deficiencies

Pathophysiology

Deficiencies of other coagulation factors that play a role in thrombin generation, cross-linking, and stabilization of the fibrin clot or down-regulation 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 most often is related to the factor levels, with the exception of FXI deficiency, in which case even patients with severe deficiencies may exhibit a variable bleeding tendency. Although FVIII and FIX deficiency are defined as rare disorders affecting <200,000 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 journal *Hemophilia* (volume 14, issue 6, November 2008).

Etiology

As with hemophilia, rare factor deficiencies can result from a genetic defect or can be due to an acquired condition. The genes for these coagulation factors are located on somatic chromosomes. Affected individuals may be homozygous or compound heterozygotes. Because the number of genetic

mutations causing most of these rare disorders may be large, the ability to predict a level or phenotypic presentation is difficult.

Acquired factor deficiencies may be associated with a wide range of conditions, including commonly encountered liver dysfunction and uncommon circumstances, such as acquired FV deficiency due to exposure to bovine thrombin. Acquired disorders may result in multiple-factor deficiencies, as seen in liver dysfunction and vitamin K deficiency, or in single-factor deficiencies, such as in amyloid-associated FX deficiency.

Each acquired clotting factor deficiency may result from a wide range of disorders, and it is beyond the scope of this chapter to review all conditions that may result in any specific coagulation disorder. The more frequently encountered disorders and associated coagulation deficiencies will be highlighted. Hypofibrinogenemia can result from 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 lead to multiple coagulation factor deficiencies. FII, FVII, FIX, and FX are vitamin K dependent and are synthesized in the liver and thus become deficient in liver failure, with vitamin K deficiency, and with the use of vitamin K antagonists. A deficiency of FII due to specific factor antibody has been observed as part of the antiphospholipid syndrome. FX deficiency may occur with amyloidosis because of adsorption of the clotting factor onto the abnormal accumulated amyloid. A deficiency of FV may occur due to cross-reacting antibody development after exposure to topical thrombin or after the use of antimicrobials, such as cephalosporins. Acquired specific coagulation factor autoantibodies have been reported for other coagulation factors outside of FVIII, but these are exceedingly rare.

Two genetic multiple-factor deficiencies occur, including combined FV and FVIII and combined vitamin K-dependent coagulation factor deficiency. Combined FV and FVIII deficiency results from mutations in two genes *LMAN1* and *MCFD2* that encode for a protein complex that functions as a cargo receptor transporting FV and FVIII from the ER to the Golgi. The combined vitamin K coagulation factor deficiency is due to a number of mutations in genes that encode for enzymes involved in the vitamin K pathway. Both conditions are rare and have been reported in consanguineous families or individuals from closed small genetic groups. These combined coagulation factor deficiency states commonly are associated with moderate to severe deficiencies and variable bleeding symptoms.

Clinical presentation

The clinical presentation of the congenital rare factor deficiencies is variable and depends on the specific clotting factor and level of deficiency. These deficiency states may be

discovered as a result of a known family history, although this is less common in autosomal recessive disorders unless a sibling has been identified. More commonly, affected individuals present with excessive bleeding, ranging from mild mucocutaneous bleeding to catastrophic intracranial hemorrhage. Unique features for each factor deficiency can be found in Table 9-6. Age at presentation is variable and most often is related to the affected coagulation factor and level of deficiency, with severe disorders presenting in childhood, and mild disorders presenting upon hemostatic challenges, such as surgeries. Patients with severe FXIII deficiency may present in the newborn period with significant umbilical stump or intracranial hemorrhage, whereas patients with severe FXI deficiency may present as adults either due to an abnormal aPTT obtained before a planned procedure or due to bleeding associated with trauma or surgery.

Acquired rare factor deficiencies present in the context of selected disorders, although these may not always be apparent during the initial presentation of the bleeding disorder. For example, patients with liver disease-associated coagulopathy often have signs and symptoms of liver dysfunction, including jaundice, ascites, and caput medusa, among others. Vitamin K deficiency may be seen in newborns who did not receive vitamin K at birth or those with malabsorption-related conditions. The resultant bleeding symptoms are similar to those seen in congenital factor deficiencies, although hemorrhagic disease of the newborn is associated with a high rate of intracranial hemorrhage.

Association of factor levels with disease severity

A recent communication of the Scientific Subcommittee of Rare Bleeding Disorders of the International Society of Thrombosis and Hemostasis underscores the importance of the association between coagulation factor levels and clinical bleeding in selected rare bleeding disorders. A thorough review of the literature and an extensive report from the known registries showed a clear correlation of undetectable levels of fibrinogen, FII, FV, and FXIII and severe bleeding. It also showed that levels <10% are associated with severe bleeding in FVII and FX deficiency. As previously reported, there was no correlation between FXI levels and spontaneous bleeding, but patients with levels < 20% appear to be at higher risk for postoperative bleeding.

Diagnosis

Once suspected, the diagnosis of a rare factor deficiency depends on the previously discussed principles of clinical history, physical examination, and an ordered systematic approach to laboratory evaluation. The majority of these deficiencies, when present at a severe or moderate level, result in

Table 9-6 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 (<0.1 g/L-1)	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 association between levels bleeding)	Surgery or injury related	None	FFP FXI concentrates available in some countries	Autoantibodies (rare)
Factor XIII (undetectable)	Intracranial Umbilical stump	Poor wound healing Miscarriage	pdFXIII concentrate: Corifacit Cryoprecipitate	Cardiopulmonary bypass Inflammatory bowel disease

RiaStap licensed for congenital afibrinogenemia. Recombinant factor VIIa is licensed for the treatment of congenital FVII deficiency. Corifacit 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.

* Official Communication of the Scientific Subcommittee on Rare Bleeding Disorders of the International Society of Thrombosis and Haemostasis (ISTH).

DIC = disseminated intravascular coagulation; FFP = fresh frozen plasma; PCC = prothrombin complex concentrate.

prolongation of the PT or aPTT. Important exceptions include deficiencies of FXIII, PAI-1, or α_2 AP, in which case the PT and aPTT are normal. The section on fibrinolysis addresses PAI-1 and α_2 AP deficiency. On the basis of the results of these screening tests and subsequent mixing studies suggesting a factor deficiency, specific factor assays are performed and may result in diagnosis. If the screening tests are not prolonged, but the clinical history is suggestive of a bleeding disorder, then specific factor assays should be performed for both deficiencies that are known to be associated with normal screening tests and for others that, when present at a mild level, may not prolong these tests. FXIII deficiency is diagnosed via a qualitative assay (clot solubility assay) or via a quantitative assay. The clot solubility assay is abnormal when the FXIII level is <5% and therefore is not consistently sensitive to mild deficiencies; at this time, the clinical phenotype of mild FXIII deficiency is not well described. If suspicion exists that the deficiency is due to an autoantibody, mixing studies will reveal the presence of a time- or temperature-dependent inhibitor.

Treatment

For patients with congenital factor deficiencies, the mainstay of therapy is replacement of the deficient coagulation factor either after bleeding occurs or as prophylactic therapy, as described for severe hemophilia. Table 9-6 lists presently available therapies for factor replacement in the United States. For the majority of patients with rare disorders, standard therapy consists of treatment when bleeding occurs or before procedures or interventions. There are important exceptions to this approach; because severe deficiencies of FX and FXIII frequently result in catastrophic intracranial hemorrhage, these patients receive lifelong prophylaxis. For FX deficiency, this is accomplished through twice-weekly infusions of a prothrombin complex concentrate, whereas for FXIII, this is accomplished via monthly infusions of a plasma-derived FXIII concentrate currently licensed and available in the United States (a recombinant FXIII is in a phase III clinical trial). Severe FVII deficiency may be

associated with intracranial hemorrhage; the clinical phenotype of severe FVII deficiency is more variable than either FXIII or FX deficiencies, therefore, prophylactic treatment should be considered based on patient and family history. Recombinant activated FVIIa is licensed in the United States for the treatment of FVII deficiency.

The approach to management of acquired rare factor deficiencies includes both treatment of bleeding and treatment of the associated condition, if present. Treatment may be as relatively simple as administration of vitamin K in vitamin K deficiency, or it can be complicated as in some cases of liver failure. For patients in whom an associated condition is not identifiable or when present, its treatment is not feasible, the goal of therapy is aimed at intervention for bleeding episodes through either nonspecific therapies, such as fresh frozen plasma, or the use of specific factor concentrates as listed in Table 9-6. An individual approach for each patient's situation and diagnosis is required. Adjuvant therapies, including antifibrinolytic and topical agents, may be used depending on the clinical circumstance. Desmopressin does not have documented efficacy in these rare deficiencies.

Prognosis and outcomes

Congenital rare factor deficiencies are highly heterogeneous conditions both within and between each disorder. Furthermore, acquired conditions that result in rare factor deficiencies are quite varied: An acquired inhibitor may require specific intervention aimed at ablation or may spontaneously remit, as seen with FV antibodies associated with thrombin use; other associated conditions, such as liver failure, may have significant morbidity or mortality. Therefore, prognosis and outcome are related to the specific deficiency, its cause, the availability of an adequate replacement product, and the clinical circumstances. In general, mild to moderate congenital rare factor deficiencies often do not result in major sequelae, and the associated bleeding may be manageable. In those with a severe congenital deficiency, particularly if associated with serious bleeding complications, prophylactic therapy may be an effective approach, if a replacement product is available. These patients may then experience improved outcomes if permanent sequelae resulting from bleeding have not yet occurred. For patients with acquired rare factor deficiencies, outcomes may range from excellent to poor. Those who recover from an underlying condition that caused the coagulopathy may have an excellent outcome if a catastrophic bleed has not occurred. For those whose underlying condition is not treatable, prognosis may be poor and often related to consequences of the underlying disorder, although bleeding may contribute to outcome.

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.

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, three 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 prothrombin complex concentrates for deficiencies of FX and FII. In FV and FXI deficiency, fresh frozen plasma remains the mainstay of therapy; in addition, platelet transfusions are sometimes used in FV deficiency as 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 with 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 disseminated intravascular coagulation (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 or as a result of a congenital deficiency; 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 2–6 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 avail-

able 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 as 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, two 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 it resistant to activation by tissue or urine plasminogen activators. Thus, these agents are effective in tissues rich in tPA or urine plasminogen activator. 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 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 rare patients 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 are acquired most often, 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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Disorders of platelet number and function

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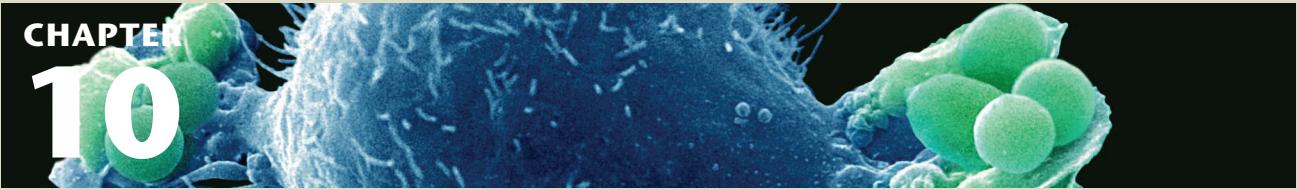
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CHAPTER
10



Disorders of platelet number and function

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Platelet biology: structure and function

Hemostasis encompasses a series of interrelated and simultaneously occurring events involving the blood vessels, platelets, and coagulation system. Defects affecting any of these major participants may lead to a hemostatic defect and a bleeding disorder. This chapter will focus on the 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 erythrocytes. Platelet volume is approximately 7 fL. Electron microscopy reveals a fuzzy coat (glycocalix) on the platelet surface composed of membrane 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; the 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 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 the high electron density), adenosine triphosphate (ATP), adenosine diphosphate (ADP), magnesium, and serotonin (5-hydroxytryptamine). Serotonin is taken up by platelets from plasma and incorporated into the 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 [PDGF], vascular endothelial growth factor [VEGF]); 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 granules from plasma.

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Off-label drug use: Dr. Rao: Desmopressin for management of patients with inherited platelet function defects and renal failure. Recombinant VIIa for management of patients with inherited platelet function defects. Dr. McCrae: Rituximab for ITP and TTP.

Platelet function in hemostasis

Following injury to the blood vessel, platelets adhere to exposed subendothelium by a process (adhesion) that involves, among other events, the interaction of a plasma protein, vWF, and a specific glycoprotein (GP) complex on the platelet surface, GP Ib-IX-V (GPIb-IX) (Figure 10-1). This interaction is particularly important for platelet adhesion under conditions of high shear stress. Adhesion is followed by recruitment of additional platelets that form clumps, a process called aggregation (cohesion). This involves binding of fibrinogen to specific platelet surface receptors, a complex composed of GPIIb-IIIa (integrin $\alpha IIb\beta 3$). 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. Activated platelets release the contents of their granules (secretion), including ADP and serotonin from the dense granules, which causes the recruitment of additional platelets. Moreover, platelets play a major role in coagulation mechanisms; several key 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

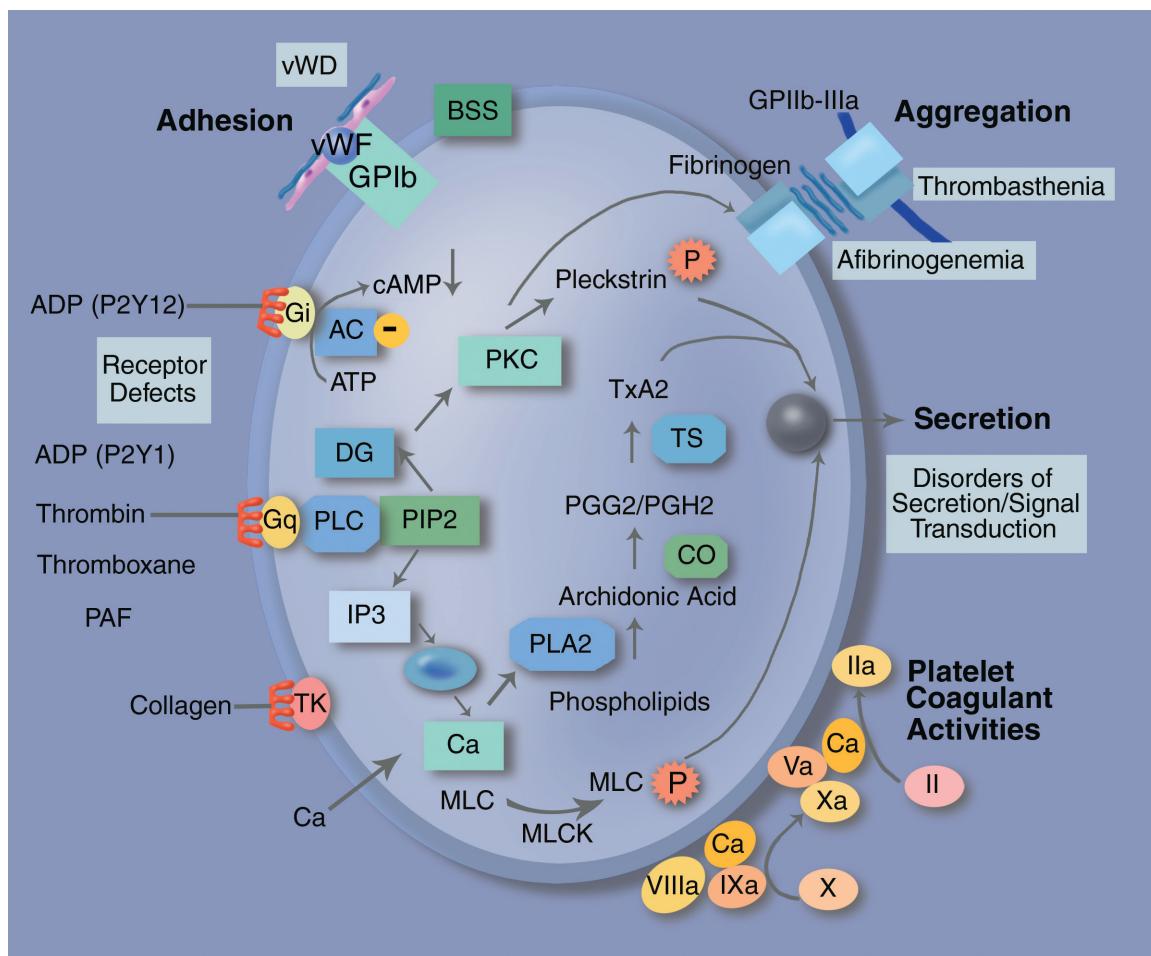


Figure 10-1 Schematic representation of selected platelet responses to activation and inherited disorders of platelet function. The Roman numerals in the circles represent coagulation factors. Modified with permission from Rao AK. Congenital disorders of platelet function: disorders of signal transduction and secretion. *Am J Med Sci*. 1998;316:69-76. AC = adenylyl cyclase; ADP = adenosine diphosphate; BSS = Bernard-Soulier syndrome; 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; PKC = protein kinase C; PLC = phospholipase C; PLA2 = phospholipase A2; TK = tyrosine kinase; TS = thromboxane synthase; TxA2 = thromboxane A2; vWF = von Willebrand factor; vWD = von Willebrand disease.

production or release of several intracellular messenger molecules, including products of hydrolysis of phosphoinositide (PI) by phospholipase C (diacylglycerol and inositol 1,4,5-triphosphate [InsP_3]), TxA_2 , and cyclic nucleotides (cyclic adenosine monophosphate) (Figure 10-1). These induce or modulate the various platelet responses of Ca^{2+} 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 binds and are modulated by guanosine triphosphate (G proteins). As in most secretory cells, platelet activation results in an increase in cytoplasmic ionized calcium concentration; InsP_3 functions as a messenger to mobilize Ca^{2+} from intracellular stores. Diacylglycerol activates protein kinase C (PKC), and this results in the 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, also are triggered by platelet activation. Either inherited or acquired defects in these platelet mechanisms may lead to impairment of the platelet role in hemostasis.

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. 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 disorders such as immune thrombocytopenia, however, although the platelet count is low, platelet life span is diminished and the megakaryocyte mass may be expanded. 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%-15% of the 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 (IL-3), interleukin 6 (IL-6), and interleukin 11 (IL-11), are required for optimal megakaryocyte development.

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 7-10 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 $1\text{-}3 \times 10^{11}$ platelets per day, although production can increase tenfold during times of high demand. The number of circulating platelets is regulated by TPO, which binds to megakaryocytes and hematopoietic stem cells via c-Mpl. c-Mpl 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 also is expressed on platelets, which bind and clear TPO from the circulation. TPO is secreted constitutively from the liver, and although its production from liver and bone marrow may

Normal platelet production

Megakaryocyte proliferation and differentiation involves 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. Each megakaryocyte produces 1,000-3,000 platelets before the remaining nuclear material is phagocytosed by resident macrophages. Released platelets circulate for 7-10 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; thus TPO levels are regulated by the platelet and megakaryocyte mass through binding of TPO to its receptor, c-Mpl.
- TPO levels are normal in immune thrombocytopenia (ITP) because of enhanced clearance of TPO bound to platelets but are elevated in bone marrow failure syndromes.
- The normal platelet life span is 7-10 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 for 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. He is in otherwise good health other than 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 looks well but several 2.0 cm bruises are noted on the distal upper extremities and back of the hands. Complete blood count reveals a hemoglobin of 12.8 gm/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 recently 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 necessarily present. Thus, the working group recommended the term *immune thrombocytopenia*, although the abbreviation ITP is preserved. In this classification scheme, *primary* is used to denote ITP with no precipitating cause, while *secondary ITP* refers to all other forms of immune-mediated thrombocytopenia (Table 10-1). These recommendations are consistent with the guidelines developed by the American Society of Hematology working group.

ITP is a common cause of thrombocytopenia in adults and children. Estimates of prevalence vary widely, ranging between 3 and 20 per 100,000 persons, with an estimated incidence of 2-10 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. 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. In most children, ITP is self-limited and often is detected after an antecedent viral or infectious illness, whereas approximately 90% of cases of ITP in adults become persistent or chronic and cannot be linked to an obvious precipitating event. Although patients with more severe thrombocytopenia may present with mucocutaneous bleeding, those diagnosed with

Table 10-1 International Working Group proposed definitions of disease.

Primary ITP	Isolated thrombocytopenia Platelets $<100 \times 10^9/L$ No other apparent causes of thrombocytopenia No secondary cause of ITP present
Secondary ITP	All other forms of immune-mediated thrombocytopenia except primary ITP Designate with presumed cause, in parentheses, following secondary ITP (eg, secondary ITP; lupus-associated)
Phases of the disease	Newly diagnosed: within 3 months of diagnosis Persistent: between 3 and 12 months of diagnosis Chronic: lasting >12 months Severe: presence of bleeding at presentation sufficient to mandate treatment, or occurrence of new bleeding symptoms requiring additional intervention

Adapted from Rodegheiro F et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386-2393.

ITP = immune thrombocytopenia.

thrombocytopenia on a routine blood count are often asymptomatic. There is no gold-standard laboratory test for ITP, and thus, the diagnosis is made by excluding nonimmune causes of thrombocytopenia and investigating potential secondary causes.

Secondary ITP occurs in the setting of drugs, such as quinine or sulfa-containing drugs (see section on drug-induced thrombocytopenia), lymphoproliferative disorders, systemic lupus erythematosus or other autoimmune disorders, antiphospholipid antibody syndrome, and infections with hepatitis C, HIV, and *Helicobacter pylori*. Nonimmune causes of thrombocytopenia, including hypersplenism, hereditary thrombocytopenias, and type 2B von Willebrand disease (vWD), also should be included in the differential diagnosis of ITP (Table 10-2). Occasional patients with myelodysplastic syndromes 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. International guidelines recommend that a platelet count below $100 \times 10^9/L$ is required for the diagnosis of ITP,

Table 10-2 Differential diagnosis of immune thrombocytopenia.

Previously diagnosed or high risk of conditions that may be associated with autoimmune thrombocytopenia (eg, HIV, hepatitis C virus, or other infection; other autoimmune or immunodeficiency disorders; malignancy; recent vaccination)
Liver disease, including cirrhosis from any cause
Drugs (prescription or nonprescription), alcohol abuse, consumption of quinine (tonic water), environmental toxins
Bone marrow disorders, including myelodysplastic syndromes, leukemias, other malignancies, fibrosis, aplastic anemia, and megaloblastic anemia
Recent transfusions (posttransfusion purpura) and recent immunization
Inherited thrombocytopenia: thrombocytopenia-absent radii syndrome, radio-ulnar synostosis, congenital amegakaryocytic thrombocytopenia, Wiskott-Aldrich syndrome, MYH9-related disease, Type IIb von Willebrand disease, Bernard-Soulier syndrome

Adapted from Rodegheiro F et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386-2393.

because mild thrombocytopenia may occur normally in non-Caucasians, and rarely results in the development of more severe thrombocytopenia or other autoimmune disease. The most common symptom of ITP is mucocutaneous bleeding, which may manifest as petechiae, purpura, ecchymosis, epistaxis, menorrhagia, oral mucosal, or gastrointestinal bleeding. The most feared complication is intracranial hemorrhage, which occurs only rarely. Bleeding because of thrombocytopenia 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 (platelets $<20 \times 10^9/L$).

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 ITP, the remainder of the general physical examination is normal. The presence of lymphadenopathy or splenomegaly should prompt investigations for underlying infection or lymphoproliferative disease. Skeletal, renal, or neurologic abnormalities suggest a familial cause of thrombocytopenia.

Recent studies suggest that fatigue is a common symptom in patients with ITP, occurring in $>20\%$ of children and up to 40% of adults. Fatigue correlates with a platelet count $<100 \times 10^9/L$ and treatment with steroids, but not with duration of ITP, age, or gender. Fatigue usually resolves with successful treatment of ITP. Finally, epidemiologic studies suggest that ITP is associated with a relative risk of thrombosis of approximately 1.5-2.0. The mechanism of thrombosis in these individuals is not well established, although the risk of thrombosis does not correlate directly with the platelet count.

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 platelet glycoproteins, most commonly GPIIb-IIIa and GPIb-IX. These antibodies may recognize the same targets on megakaryocytes, leading to impairment of megakaryocyte proliferation and differentiation, and proplatelet production. In most patients, both enhanced platelet destruction and impaired platelet production contribute to the development of thrombocytopenia. As noted, plasma levels of TPO generally are not elevated in patients with ITP.

Dysregulated T-cells in patients with ITP may enable the development of platelet autoantibodies, have a direct cytotoxic effect on platelets, and impair platelet production by megakaryocytes. Recent interest has focused on decreased levels of regulatory T-cells (T_{reg}) in patients with ITP; successful ITP treatment has been associated with restoration of T_{reg} levels.

The pathogenesis of *secondary ITP* may share similar mechanisms as 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 the exclusion of other causes. Leukocyte counts and hemoglobin are normal unless significant

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, or abnormalities suggestive of other disorders. The mean platelet volume (MPV) may be increased in patients with ITP. Some 15%–25% of ITP patients have detectable antinuclear or antiphospholipid antibodies; these generally have no prognostic importance, although one report suggested an increased incidence of thrombosis in ITP patients with antiphospholipid antibodies.

Bone marrow examination is not required routinely, but it should be performed to exclude other causes of thrombocytopenia when atypical features such as unexplained anemia, lymphadenopathy, or splenomegaly are present. Because approximately 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. Bone marrow examination also may be warranted in elderly patients in whom myelodysplasia is suspected and should be considered in patients scheduled to undergo splenectomy. Megakaryocyte number is typically normal or increased in the marrow of patients with ITP.

With increased appreciation that secondary causes of ITP may be more common than previously believed, additional laboratory studies, such as screening for hepatitis C and HIV, and evaluation for combined variable immunodeficiency should be considered. Table 10-3 contains a list of suggested screening studies proposed by the ITP International Working Group.

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, usually in the first month after diagnosis. 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 Coombs 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 6 months but occasionally over a year or more. The remaining 20% have persistent thrombocytopenia, yet even in this group, major bleeding is uncommon. Splenectomy generally is reserved for severe persistent thrombocytopenia and 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 is another effective therapy in children, with a long-term remission rate of 22% in retrospective analyses. Thrombopoietic agents are

Table 10-3 International Working Group recommendations for the diagnosis of ITP in adults.

Basic evaluation	Test 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 (in selected patients)	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. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood*. 2010;115(2):168–186.

CBC = complete blood count; CMV = cytomegalovirus; HCV = hepatitis C virus; IgG = immunoglobulin G; ITP = immune thrombocytopenia; PCR = polymerase chain reaction; TPO = thrombopoietin.

also effective in refractory childhood ITP, although the safety of long-term treatment with these agents in the pediatric population has not been established.

Management of primary ITP in adults

In contrast to children, ITP in adults evolves into a chronic disease in approximately 90% of patients. Given this realization, the goal of ITP management in adults is to maintain a safe platelet count while minimizing the toxicity of therapy. Therapy should not be dictated by the platelet count alone, but it also should consider other factors that modulate the risk of bleeding. There are no controlled studies demonstrating the superiority of any specific sequential treatment algorithm and significant variability exists among the treatment approaches advocated by different hematologists.

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, and although there is significant variability in bleeding among individual patients, this platelet count threshold has been suggested as a cutoff for considering treatment of ITP. Although several first-line therapies are available, prednisone (1 mg/kg daily) remains the initial treatment of choice because of its efficacy and low cost. Approximately 75% of patients initially respond to corticosteroids, although tapering usually precipitates relapse, and ultimately only 10%-15% of patients are able to maintain a safe platelet count after steroid discontinuation. High-dose dexamethasone (40 mg daily for 4 days, repeated in biweekly or monthly cycles) provides an alternative for the initial treatment of patients with ITP, with some studies suggesting that high-dose corticosteroids used early in the treatment course induce more durable remissions. A still more aggressive approach that employed dexamethasone and rituximab in the initial treatment of ITP demonstrated a significantly higher sustained response rate at 6 months after treatment initiation in patients that received this combination versus those receiving dexamethasone alone (63% vs. 36%, $n = 52$, $p < 0.004$, 95% confidence interval [CI] 0.079-0.455), although these differences appear to be lost on longer term follow-up. Up to 5%-10% of patients with ITP may achieve a durable remission, usually within the first year after presentation; however, it is uncertain whether these are spontaneous or related to treatment. 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 durable response after initial treatment with corticosteroids, intermittent IVIg or anti-D may be effective. Both of these agents are associated with response rates similar to those of corticosteroids;

however, the duration of response generally is only 2-4 weeks and thus frequent, intermittent dosing is required if these agents are used as 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 safe range until more definitive therapy can be initiated.

There are several additional options for therapy after steroid failure, specifically rituximab, thrombopoietic agents, or splenectomy. Splenectomy has been a popular therapy for decades, although the availability of alternative treatments, concerns about long-term adverse events following splenectomy, and the realization that some patients with newly diagnosed ITP ultimately may improve over time has led to decreased utilization (20%-25% of patients) in contemporary cohorts compared with older series (50%-60% of patients). Although both the ITP International Working Group and the revised American Society of Hematology (ASH) guidelines consider splenectomy an acceptable second-line therapy for ITP, the former group weights splenectomy equally to more than 10 other options, whereas the ASH guidelines *recommend* splenectomy (grade 1B evidence) for patients who fail corticosteroids while *suggesting* rituximab or thrombopoietic agents (grade 2C evidence). Splenectomy leads to a high rate of durable remissions. In a systematic review, 1,731 (66%) of 2,623 adults with ITP achieved a complete response following splenectomy with a median follow-up of 28 months (range 1 to 153 months), and ~65% of patients remained in complete remission 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% for laparoscopic splenectomy and 1.0% and 12.9% for open splenectomy, an increased risk of postsplenectomy infection, and a potentially increased risk of vascular thrombosis compared with the general population (although whether this is increased compared with nonsplenectomized age-matched ITP controls is unknown). The incidence of infection may be reduced by presplenectomy vaccination; repeat immunization or monitoring of antibody titers every 5 years may further reduce infection rates. Aggressive treatment of fever in splenectomized ITP patients is indicated.

Rituximab, an anti-CD20 monoclonal antibody that rapidly depletes CD20⁺ B lymphocytes, provides another treatment option that many hematologists consider before splenectomy in patients who fail initial corticosteroid

therapy. In a systematic review of 313 ITP patients, half of whom were not splenectomized, 62.5% achieved a platelet count response (platelet increment of $50 \times 10^9/\text{L}$), 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 $\geq 50 \times 10^9/\text{L}$ with at least a doubling from baseline at 1 year, and in 33.3%, this response was sustained for 2 years. A recent pilot randomized, placebo-controlled trial that assessed a composite endpoint of any platelet count $< 50 \times 10^9/\text{L}$, significant bleeding, or rescue treatment once standard treatment was stopped failed to demonstrate a treatment advantage for rituximab within 6 months of therapy initiation. Complete responses and overall platelet responses, however, were observed in 46.2% and 73.1% of placebo treated patients, respectively. An appealing aspect of rituximab therapy is the induction of long-term responses in a subset of patients; in one recent series, 21% of adults treated with rituximab achieved treatment-free responses of at least 5 years. Adverse effects of rituximab include infusion reactions (eg, hypotension, chills, 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 for treatment.

The TPO receptor agonists romiplostim and eltrombopag are approved in the 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. The short-term safety and tolerability of these agents was demonstrated in clinical trials and confirmed through Food and Drug Administration-mandated postmarketing surveillance, although safety data beyond 5 years is only beginning to emerge. A disadvantage of these agents includes the potential need for long-term therapy, 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 TPO receptor agonists, but there is no evidence for development of progressive or irreversible bone marrow fibrosis. Eltrombopag carries a warning

because of the potential for hepatotoxicity. Occasional patients treated with either agent may develop more severe thrombocytopenia following discontinuation than existed before treatment.

Additional treatment options for refractory ITP include azathioprine, danazol, dapsone, and other immunosuppressant medications; however, evidence from randomized controlled trials of these agents is limited. Thrombocytopenia in patients with secondary ITP often responds to treatment of the underlying disease, for example, eradication of hepatitis C with antiviral therapy or treatment of HIV with highly active antiretroviral therapy (HAART). Treatment of *H. pylori* infection has led to resolution of ITP in >50% of cases in certain regions, particularly Japan, although generally it has not been effective in North America. This may reflect differences in endemic *H. pylori* strains in different geographic regions.

Emergency treatment of ITP

Patients with new-onset, severe thrombocytopenia ($< 20 \times 10^9/\text{L}$) 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 2-3 days) or IVIg (1 g/kg for 1-2 days). Increases in the platelet count may become apparent within 3-5 days, although complete responses may require 1-2 weeks. Bleeding manifestations sometimes may improve before notable improvements in the platelet count. Emergency splenectomy may be required for patients with refractory thrombocytopenia and persistent bleeding.

Key points

- ITP may exist as a primary disorder or secondary to a number of other illnesses.
- 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 ~90% of patients.
- The pathogenesis of ITP involves accelerated platelet destruction and decreased platelet production.
- Corticosteroids are first-line therapy for ITP, although rarely induce a durable remission.
- There is no universally accepted scheme for treatment of corticosteroid-resistant ITP, although rituximab, splenectomy, thrombopoietic agents, and other drugs are all effective

Drug-induced immune thrombocytopenia

More than 200 drugs have been implicated in drug-induced immune thrombocytopenia (DITP), including quinine and quinidine (present in tonic water, bitter melon, and certain medications), nonsteroidal anti-inflammatory agents, trimethoprim-sulfamethoxazole, vancomycin, rifampin, anticonvulsants, sedatives, and acetaminophen as well as the platelet GPIIb-IIIa inhibitors tirofiban, eptifibatide, and abciximab. A case-control study of drug use among patients with acute reversible thrombocytopenia compared with nonthrombocytopenic controls showed that trimethoprim-sulfamethoxazole was most frequently implicated. 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. George et al. developed an online database of implicated drugs (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 develops approximately 7 days after drug exposure, although when induced by the GPIIb-IIIa antagonists, eptifibatide, tirofiban, and abciximab may present within hours and even on the first exposure to the drug. Several mechanisms, specific for individual drugs, underlie the development of DITP. Quinine-induced thrombocytopenia was described >140 years ago and serves as a prototype. In this disorder, the binding of naturally occurring antibodies to platelet GPs is greatly enhanced in the presence of sensitizing drug. This may result from binding the drug to specific GPs, such as GPIIb-IIIa or GPIb-IX, and perhaps to the antibody itself. Affinity maturation of B-cells producing such antibodies may result in the generation of antibodies that can destroy platelets in the presence of drug. Another mechanism of DITP involves the induction of autoantibodies by drugs such as gold, procainamide, sulfonamides, 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 (DPT), and measles-mumps-rubella (MMR), 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 [MIBS])

induced in GPIIb-IIIa following mimetic binding. Abciximab, a chimeric (mouse–human) Fab fragment to GPIIb-IIIa, causes acute profound thrombocytopenia in 0.5%–1.0% of patients on their first exposure because of preexisting antibodies that recognize the murine portion of abciximab. Thrombocytopenia caused by GPIIb-IIIa antagonists may be severe, with platelet counts $<10 \times 10^9/L$. Patients may require platelet transfusions to treat hemorrhagic complications, which are exacerbated by the concomitant use of heparin, aspirin, and other antiplatelet agents.

Diagnosis of DITP

Clinical criteria for levels of evidence for DITP have been proposed that may be used to judge the likelihood of drug being implicated in DITP. These include the temporal association between drug exposure and thrombocytopenia, the exclusion of other causes of thrombocytopenia, and recurrence upon drug rechallenge. In practice, however, patients are often on many drugs and have concurrent illnesses, such as infections, that may make the diagnosis of DITP difficult. The detection of an antibody that binds tightly to normal platelets in the presence of the drug establishes the diagnosis in many cases; however, such testing is available only in specialized laboratories and results frequently are not available in time to aid with acute treatment decisions. Moreover, drug-dependent platelet antibodies may be missed when the antibodies recognize a metabolite of the drug instead of the drug itself, as with naproxen and acetaminophen.

Treatment for DITP involves discontinuation of the drug and administration of platelet transfusions for severe bleeding. Resolution of thrombocytopenia may require 4–8 days, although bleeding symptoms usually improve more rapidly. Corticosteroids or IVIg have been beneficial in anecdotal cases, although their efficacy has not been assessed in controlled studies.

Key points

- DITP is caused by many drugs.
- Quinidine, quinine, and trimethoprim-sulfamethoxazole are commonly implicated.
- 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 the demonstration of a drug (or drug metabolite)-dependent, platelet-reactive antibody 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), with a reported incidence of 0.2%-5.0%; the risk of HIT associated with low-molecular weight heparin (LMWH) is five- to tenfold lower. Thrombosis develops in 40%-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 that is uncommonly diagnosed and caused by direct platelet agglutination by heparin.

Clinical features

HIT is uncommon in patients <40 years of age and is more common in females (odds ratio 2.37). The incidence of HIT is approximately threefold greater in surgical than medical patients. Of the surgical patients, those undergoing orthopedic surgery have the highest incidence of HIT (5%); cardiac surgery patients have a lower incidence of HIT (2%-3%) despite a higher seroconversion rate in the heparin-PF4 enzyme-linked immunoadsorbent assay (ELISA). Clinical features consistent with HIT include a platelet count decrease of 50% or more that begins 5-10 days after starting heparin (or sooner in patients with recent heparin exposure), the presence of thrombosis, and the exclusion of other causes. Absolute thrombocytopenia (platelet count $<150 \times 10^9/L$) is

not required for a diagnosis of HIT; rather a decrease in the platelet count from baseline is required. Uncommonly, HIT may develop 2-3 weeks after prior heparin exposure (delayed onset HIT). Several clinical scoring systems have been developed to assist with determining the pretest probability of HIT. The most commonly used is the 4T system (thrombocytopenia, timing, thrombosis, and other; see Table 10-4). 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 also is of limited utility in intensive care patients, a setting in which HIT is uncommon. Another system, the HIT expert probability score also has been developed, although the clinical experience with this system is not extensive. The impact of either scoring system on patient outcomes has not been determined.

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. Venous thrombosis occurs twice as frequently as arterial thrombosis, although limb artery thrombosis, myocardial infarction, and microvascular thrombosis have been described. Phlegmasia due to occlusion of the lower-extremity venous system resulting in arterial insufficiency may be difficult to discern from arterial thrombosis. Adrenal infarction, skin necrosis at the heparin injection site, 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, and fistulas, and visceral vessels also may develop. HIT-associated thrombosis occurs with increased frequency at sites of vessel injury, thus vascular

Table 10-4 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 of $10-19 \times 10^9/L$	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., Evaluation of pretest clinical score (4T's) for the diagnosis of heparin-induced thrombocytopenia in two clinical settings. *J Thromb Haemost*. 2006;4:759-765.

interventional procedures and placement of intravascular devices such as vena caval filters should be avoided.

HIT testing

Two types of tests are available for detection of HIT antibodies: quantitative PF4/heparin immunoassays (PF4/heparin ELISA) and functional assays demonstrating the ability of HIT antibodies to activate platelets, such as the serotonin release assay (SRA), generally considered the gold standard for diagnosis, or heparin-induced platelet activation (HIPA).

The sensitivity of the PF4/heparin ELISA approaches 100%, and thus a negative test is useful in excluding HIT. Difficulties concerning its use include long turnaround time in institutions in which it is not performed daily, and its low specificity and positive predictive value, particularly in the postcardiac surgery setting; the latter reflects a significant incidence of false-positive results. Specificity may be increased by considering the level of positivity. High ELISA reactivity correlates closely with the presence of platelet-activating HIT IgG in some studies, whereas positive platelet activation studies were uncommon in patients with weakly positive ELISA values (0.4–0.9). The use of an ELISA that detects only anti-PF4/heparin IgG as opposed to the polyclonal ELISA that detects IgG, IgA, and IgM antibodies also may increase specificity, as may the addition of a confirmatory step performed in the presence of high heparin concentrations.

Functional assays have improved specificity compared with the ELISA. These assays are technically difficult, however, requiring washed donor platelets, and for the SRA, radioisotope. Because of these considerations, the performance of functional assays is limited primarily to specialized reference labs, and their results generally are not available at the time the diagnosis of HIT must be considered.

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 2–3 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 10-5).

The cornerstone of HIT therapy is immediate discontinuation of heparin when the disease is suspected, usually before laboratory diagnosis. All individuals with suspected HIT should receive ultrasound evaluation of the extremities.

Table 10-5 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. Treatment and prevention of heparin-induced thrombocytopenia. *Chest*. 2012;141(2)(suppl):e495S-e530S. HIT = heparin-induced thrombocytopenia; LMWH = low-molecular weight heparin.

Anticoagulation using a nonheparin anticoagulant should be initiated even in patients with no thrombosis because of the continued high risk of thrombosis after heparin discontinuation. Alternative anticoagulation should be continued until the platelet count has normalized; some advocate for a longer duration of anticoagulation (eg, 30 days), although no controlled data demonstrating the benefit of this approach are available. Patients with HIT and no thrombosis should receive at least 1 month of full-dose anticoagulation, and those with thrombosis should receive at least 3 months. LMWH should not be used because of cross-reactivity with most heparin-dependent antibodies. Initiation of warfarin without coverage by an alternative anticoagulant may lead to hypercoagulability because of the inhibition of protein C γ-carboxylation, and patients who develop HIT while on warfarin or who have been started on warfarin alone should be treated with vitamin K in addition to a nonheparin anticoagulant.

Currently available nonheparin anticoagulants available in the United States include argatroban and bivalirudin, both of which are direct thrombin inhibitors. Argatroban is hepatically cleared and approved for treatment of HIT with or without thrombosis, as well as percutaneous coronary interventions 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 in conjunction with warfarin, it may have significant effects on the PT. Thus, transitioning patients from argatroban to warfarin should be performed 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. Other anticoagulants such as Danaparoid and Lepirudin are no longer available in the United States. A number of reports have described the use of the synthetic pentasaccharide fondaparinux in patients with HIT, although this agent has not been studied in a controlled manner.

Key points

- HIT occurs in 0.2%-5% of adults exposed to UFH, approximately 40% of whom develop thrombosis.
- HIT antibodies are directed against a large, multimolecular complex of PF4/heparin.
- ELISA tests for HIT antibodies are highly sensitive, but they have low specificity and thus are frequently positive when confirmatory functional assays, including the ¹⁴C-serotonin release assay, are not.
- When HIT is suspected, heparin must be discontinued and a nonheparin anticoagulant initiated.
- Anticoagulation with a nonheparin anticoagulant generally is continued for 30 days in patients with HIT without thrombosis and for at least 3 months in those who develop thrombosis.
- Delayed-onset HIT may develop several weeks after heparin exposure.

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 hemoglobin of 8.5 gm/dL, 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. Peripheral blood film reveals 1-2 schistocytes per high-power field. Subsequent evaluation included sequencing of complement regulatory genes and revealed a mutation in factor H.

Clinical features

The thrombotic microangiopathies discussed in this chapter include thrombotic thrombocytopenic purpura (TTP) and the typical and atypical hemolytic uremic syndrome (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 MAHA and thrombocytopenia without another apparent cause is sufficient for the diagnosis of thrombotic microangiopathy (TMA).

TTP occurs in both a rare inherited form due to 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. aHUS may present in a similar manner, but it also may demonstrate a more chronic presentation with progressive renal insufficiency, low-grade MAHA, and thrombocytopenia. Typical HUS follows infection with enteropathogenic *E. coli*, may occur in epidemics, and often is preceded by bloody diarrhea and abdominal pain (the frequent presence of diarrhea has led to the designation of this disorder as D⁺, as opposed to aHUS, referred to as D⁻). 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 these TMAs may be difficult because of extensive overlap in symptoms. Although some features, such as neurologic manifestations may be more frequent in TTP, renal failure is more common in HUS and aHUS; however, these characteristics alone do not allow discrimination. Because of these overlapping symptoms, it has been difficult to develop unambiguous classification schemes for these disorders. Recent scientific advances have led to new information concerning the pathogenesis of these diseases. For example, although most cases of TTP are associated with deficiency of ADAMTS13, activation of the alternative pathway of complement resulting from mutations in complement regulatory proteins underlie approximately 70% of cases of atypical aHUS. These differences have allowed for 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 10-6.

Pathogenesis

TMAs cause microvascular thrombi in critical organs, leading to ischemia and organ damage. These thrombi induce

Table 10-6 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 and verotoxin (Shiga-like toxin) producing bacteria

Streptococcus pneumoniae

Defective cobalamin metabolism

Quinine induced

Disorders in which etiology is not well understood

HIV

Malignancy

Drugs

Pregnancy

Systemic lupus erythematosus and antiphospholipid antibody syndrome

Adapted from Taylor CM et al. Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom. *Br J Haematol*. 2009;148:37-47.

ADAMTS13 = a disintegrin and metalloprotease with thrombospondin.

shearing of red blood cells, leading to the characteristic schistocytic anemia, which also may be caused by oxidative stress. Endothelial cell activation or damage also promote TMA, leading to the elaboration of unusually large vWF multimers that enhance platelet agglutination and microvascular occlusion.

Most cases of TTP result 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 10-2). The observations that some patients develop apparent TTP despite normal levels of circulating ADAMTS13, whereas other patients with congenital ADAMTS13 deficiency may not develop TTP until adulthood, suggests that factors other than ADAMTS13 deficiency, such as endothelial damage or activation, also are needed to trigger TTP. Other TTP-like syndromes can be caused by drugs, including quinine, ticlopidine, clopidogrel, cyclosporine, tacrolimus, mitomycin C, and gemcitabine, and also 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.

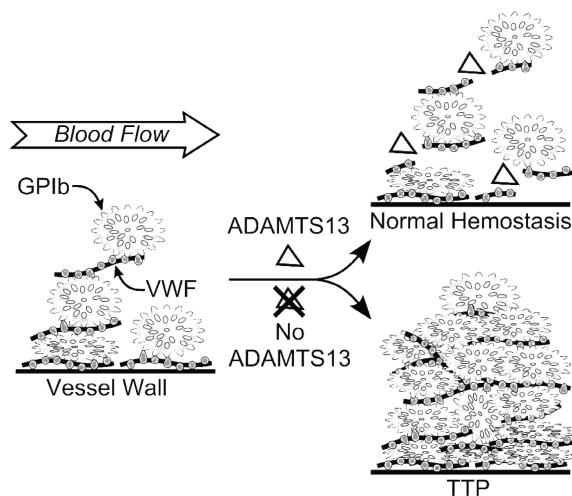


Figure 10-2 Pathogenesis of idiopathic TTP caused by ADAMTS13 deficiency. Multimeric vWF adheres to endothelial cells or to connective tissue exposed in the vessel wall. Platelets adhere to vWF through platelet membrane GPIb-IX. In flowing blood, vWF in the platelet-rich thrombus is stretched and cleaved by ADAMTS13, limiting thrombus growth. If ADAMTS13 is absent, vWF-dependent platelet accumulation continues, eventually causing microvascular thrombosis and TTP. Reproduced from Sadler JE. von Willebrand factor, ADAMTS-13 and thrombotic thrombocytopenic purpura. *Blood*. 2008;112 (1):11-18. ADAMTS13 = a disintegrin and metalloprotease with thrombospondin; GP = glycoprotein; TTP = thrombotic thrombocytopenic purpura; vWF = von Willebrand factor.

HUS results from infection by enteropathogenic *E. coli*, most commonly serotype O157:H7. The capacity of these organisms to cause HUS reflects their production of two 70 kD bacterial exotoxins named 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 kD Stx holotoxin consists of a 32 kD A subunit and five 7.7 kD B receptor-binding subunits that bind globosyltriaosylceramide (Gb3; CD77) receptors expressed on capillary endothelium. Following binding to Gb3, the toxin is internalized. The A subunit is proteolyzed to a 27 kD 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 resulting in loss or functional impairment of complement regulatory proteins, or less frequently, activating mutations in complement proteins themselves. aHUS is transmitted in an autosomal manner, accounting for the familial inheritance, although penetrance is only 50%. Under normal conditions, the AP is constitutively activated because of ongoing C3 hydrolysis (Figure 10-3), 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%-10% of cases of aHUS result from acquired antibodies to factor H. Mutations in MCP, 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%-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 can be assumed in patients with MAHA and thrombocytopenia without another apparent etiology, such as malignant hypertension, vasculitis, scleroderma renal crisis, tumor emboli, or disseminated intravascular coagulation. Fever and neurologic symptoms are less common, although evidence of renal involvement even in the absence of renal insufficiency sometimes can be obtained through examination of the urinary sediment. Schistocytes are invariably present and are accompanied by elevation of the LDH, which may be striking; levels of unconjugated bilirubin also may be increased. Nucleated red blood cells frequently are present. The PT, PTT, and fibrinogen levels are 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 often presents during pregnancy, particularly in the second and third trimesters. ADAMTS13 assays may be useful in confirming the diagnosis of TTP when severe deficiency (<5%) is present in the appropriate clinical setting, and provide prognostic information, with lower levels of ADAMTS13 and higher levels of anti-ADAMTS13 antibodies associated with higher relapse rates. Many patients with TMA 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

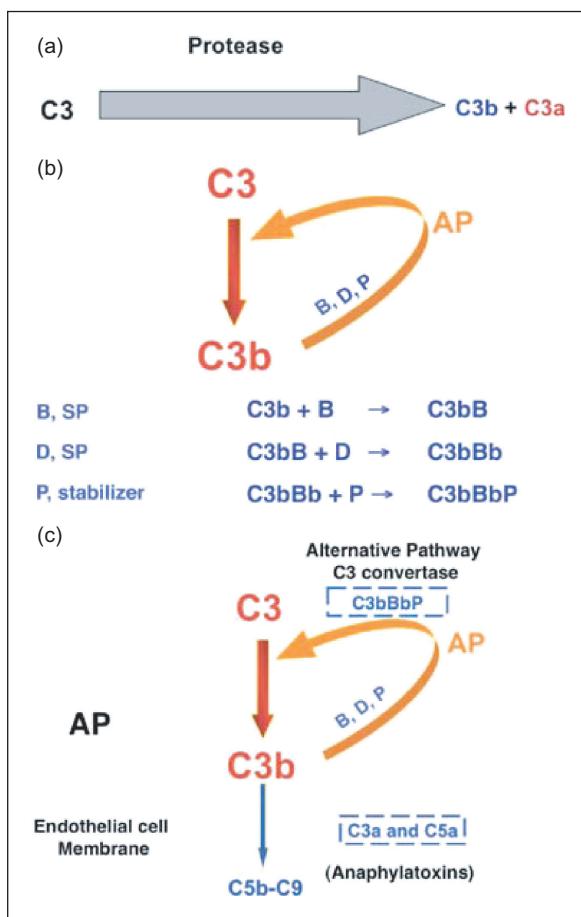


Figure 10-3 The alternative pathway of complement activation. (a) The AP of the complement system originally consisted of a serine protease that cleaved C3 to the opsonin C3b and the proinflammatory anaphylatoxin C3a. (b) An amplification loop was next evolved to more efficiently deposit C3b on a target 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). Reproduced from Liszewski MK, Atkinson JP. Too much of a good thing at the site of tissue injury: the instructive example of the complement system predisposing to thrombotic microangiopathy. *Hematology: Am Soc Hematol Educ Program*. 2011;2011:9-14. AP = alternative complement pathway; GP = glycoprotein.

plasma exchange may lag behind clinical responses and are not useful in determining the duration of plasma therapy.

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 may make such a history difficult to dissect. Exacerbations of disease may follow upper-respiratory infections and may be accompanied by fatigue and malaise. aHUS commonly presents in association with pregnancy, most commonly at 3–4 weeks postpartum. Complement levels in patients with aHUS may be decreased, but normal levels do not exclude aHUS. Sequencing of complement inhibitor proteins, factors B or C3, are useful in confirming a clinical impression of aHUS, but this sequencing is performed only in specialized laboratories and therefore not useful in the acute setting.

Typical HUS is the most common cause of acute renal failure in children and is most common in the pediatric population. This disorder, however, increasingly has been recognized in adults. The disease begins with abdominal pain and watery diarrhea 2–12 days after toxin exposure. This presentation may be difficult to differentiate from inflammatory bowel disease, appendicitis, ischemic colitis, or intussusception. 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 definitive diagnosis is made by culture of *E. coli* O157:H7 on sorbitol-MacConkey agar. The presence of Shigatoxin or its structural genes may be detected by enzyme immunoassay or PCR of the stool. Serologic studies demonstrating an increase in convalescent antibody titer to Shigatoxin 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 to 1.5 plasma volumes is standard initial treatment; however, larger volume exchanges may have additional benefit in patients with an inadequate response. Plasma exchange is continued daily until the platelet count reaches normal levels ($>150 \times 10^9/L$), LDH normalizes, 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 use of rituximab in patients who did not respond rapidly to plasma exchange (with plasma exchange continued), 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 vincristine, as well as splenectomy, which may decrease relapse rates. Platelet transfusion has been associated with a rapid decline in clinical status in occasional patients, although a retrospective analysis could not identify a clear association of platelet transfusion with poor outcomes.

Plasma exchange historically has been the treatment of choice for aHUS as well as TTP, and it remains so in patients with TMA in whom a clear diagnosis of TTP or aHUS cannot be established. 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. Thus, in established aHUS, eculizumab may be the treatment of choice, though its role in plasma exchange refractory TMA and related syndromes has not been established.

Treatment of *E. coli*-associated typical HUS is generally supportive; the use of antibiotics may lead to increased toxin release and should be avoided. Some patients may require dialysis during the acute phase of their illness. A benefit for plasma exchange in typical HUS has not been demonstrated. The use of Eculizumab in this disorder is under investigation.

Key points

- TTP, atypical HUS (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 that neutralize ADAMTS13 activity. This leads to accumulation of ultralarge vWF multimers that induce platelet agglutination in the microcirculation.
- The pathogenesis of most cases of aHUS involves excessive activation of the AP, leading to complement-mediated damage to vascular cells.

Key points (continued)

- The pathogenesis of typical HUS reflects the toxic effects of Shiga toxin on vascular endothelium and other cell types.
- The treatment of choice for TTP is plasma exchange.
- Plasma exchange is effective in some cases of aHUS, although therapies aimed at inhibition of activation of the AP (ie, Eculizumab) appear more effective.
- Plasma exchange is not effective in typical HUS, which is usually self-limited.
- Pregnancy is associated with an increased frequency of TTP (in the second and third trimesters) and aHUS (postpartum).

Splenic sequestration

Splenic enlargement, usually from advanced liver disease or cirrhosis, 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 $60-100 \times 10^9/L$. Other mechanisms associated with liver disease that may induce thrombocytopenia include viral hepatitis-induced secondary ITP, suppression of platelet production by megakaryocytes resulting from direct viral infection, and decreased production of TPO by the cirrhotic liver. Therapy of chronic hepatitis with interferon- α also may induce thrombocytopenia.

Familial thrombocytopenia

Familial thrombocytopenic syndromes are uncommon, and patients often are misdiagnosed as having ITP. Recognition of these disorders is important to avoid unnecessary and potentially harmful treatments. The diagnosis should be considered in any patient with a family history of thrombocytopenia, or in patients with “ITP” who do not respond to standard therapy. The presence of anatomic defects, including absent radii (thrombocytopenia-absent radii [TAR] syndrome) or right-heart defects (DiGeorge syndrome), and laboratory features, including large platelets and neutrophil inclusions on the blood film (as seen in the *MYH9*-related disorders), support the diagnosis of familial thrombocytopenia.

Autosomal dominant *MYH9*-related macrothrombocytopenic disorders are caused by mutations in the *MYH9* gene, which codes for nonmuscle myosin IIA. These include May-Hegglin, Fetchner, Sebastian, and Epstein syndromes. Associated features include large platelets, Döhle bodies in neutrophils (Figure 10-4), renal failure, hearing loss, and cataracts. Bernard-Soulier syndrome (BSS) is an autosomal recessive familial thrombocytopenic disorder characterized by the absence of the platelet GPIb-IX complex that is associated with large platelets, lack of platelet aggregation by high-dose ristocetin, and bleeding. Wiskott-Aldrich syndrome

(WAS) is an X-linked disorder characterized by severe immunodeficiency, small platelets, and eczema. Congenital amegakaryocytic thrombocytopenia (CAMT) is a recessive disorder characterized by severe thrombocytopenia and absence of megakaryocytes in the bone marrow that results from mutations in the Mpl receptor, and may lead to trilineage failure. Inherited thrombocytopenias also occur in association with mutations in specific transcription factors that regulate megakaryocyte and platelet production, including GATA1 (sex-linked inheritance) and RUNX1 (autosomal dominant). Patients with the Paris-Trousseau/Jacobsen syndrome, an autosomal dominant macrothrombocytopenia, have psychomotor retardation and facial and cardiac abnormalities; this syndrome arises because of the deletion of a portion of chromosome 11, 11q23-24, that encompasses the gene encoding the transcription factor friend leukemia integration 1 (FLI-1).

Establishing the diagnosis of familial 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 non-muscle 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, and several laboratories in the United States and Europe now provide these services (see <http://www.genetests.org>).

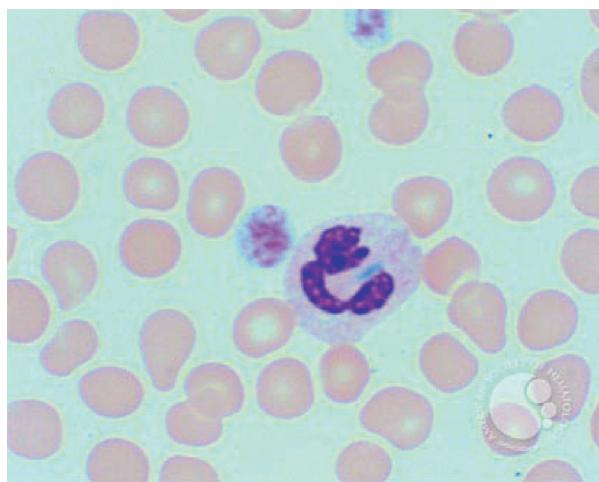


Figure 10-4 May-Hegglin anomaly, an *MYH9*-associated disorder. This peripheral blood film demonstrates a giant platelet, the size of which is similar to neighboring red blood cells. Immediately adjacent is a neutrophil containing a blue Döhle body between the nuclear lobes. Döhle bodies also are seen in infection, where they are located more commonly in the cell periphery, as opposed to the one seen in this figure. From ASH Image Bank #00003385 (submitted by Julia Braza).

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 an inherited thrombocytopenia.
- Genetic diagnosis of inherited thrombocytopenia should be obtained when possible.

setting is extensive and includes drugs, infection, and immune disorders, including ITP, DIC, and TMAs, among others. No high-quality evidence is available to guide the management of such patients, but acutely ill individuals with thrombocytopenia often require platelet transfusion. The optimal threshold for platelet transfusion in this population is uncertain, but platelets generally are given when the platelet count decreases to $\sim 20 \times 10^9/L$. Bleeding or a planned invasive procedure should prompt consideration of platelet transfusion at a higher platelet count.

Infection-associated thrombocytopenia

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. Thrombocytopenia commonly is associated with HIV and hepatitis C virus infection, both causes of secondary ITP. In HIV, thrombocytopenia usually responds to treatment with highly active antiretroviral therapy.

A rare and unusual manifestation 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 occur in response to infection, with Epstein-Barr virus (EBV) being the most common cause. 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, but usually in the context of bycytopenias and pancytopenia. Diagnosis rests on meeting specific clinical criteria, as well as markedly elevated levels of ferritin and the circulating α -chain of the interleukin-2 (IL-2) receptor. Demonstration of hemophagocytosis on tissue or bone marrow biopsies is useful but not required. Therapy is directed toward eradication of EBV-infected cells, generally using multiagent approaches.

Thrombocytopenia in the critically ill

This topic is covered in greater detail in Chapter 1. Approximately 40% of patients in medical or surgical intensive care units (ICUs) develop a platelet count $<150,000/\mu L$; 20%-25% develop a platelet count $<100,000/\mu L$, and 12%-15% develop severe thrombocytopenia, with a platelet count $<50,000/\mu L$. The development of thrombocytopenia in patients in the ICU is a strong independent predictor for ICU mortality. The spectrum of disorders that cause thrombocytopenia in this

Key points

- Infection is a common cause of thrombocytopenia, particularly in ICU patients, that can be induced by a variety of organisms.
- The diagnosis of HLH is based on clinical criteria, as well as elevated levels of ferritin and the circulating IL-2 receptor α -chain

Disorders of platelet function

Disorders of platelet function are characterized by highly variable mucocutaneous bleeding manifestations and excessive hemorrhage following surgical procedures or trauma. Spontaneous hemarthrosis and deep hematomas are unusual in patients with platelet defects. In general, most patients have mild to moderate bleeding manifestations. A majority of patients, but not all, have a prolonged bleeding time. 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. The platelet dysfunction in these patients arises by diverse mechanisms.

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 $190,000/\mu L$, and the hemoglobin is 11 g/dL . The bleeding time is prolonged at 14 minutes (normal range, 3-7 minutes), and plasma levels of factor VIII, vWF antigen, and ristocetin cofactor are within normal range. Previous blood work had demonstrated normal platelet counts. 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 hematologist discusses the diagnosis and management with the parents.

Table 10-7 provides a classification of inherited disorders associated with impaired platelet function, based on the platelet function or responses that are abnormal (Figure 10-1). Of note, not all of these disorders are due to a defect in the platelets per se. Some, such as vWD and afibrinogenemia, result from deficiencies of plasma proteins essential for platelet aggregation or adhesion. Some of these disorders are distinctly rare, but they shed enormous light on platelet physiology. Moreover, in many patients with inherited abnormalities in platelet aggregation responses, the underlying molecular mechanisms remain unknown. In patients with defects in platelet–vessel wall interactions (adhesion disorders), adhesion of platelets to subendothelium is abnormal. The two disorders in this group

are vWD, resulting from a deficiency or abnormality in plasma vWF, and BSS, in which platelets are deficient in GPIb (and GPV and GPIX); in both disorders, platelet–vWF interaction is compromised. Binding of fibrinogen to the GPIb-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 GPIb-IIIa complex, which binds fibrinogen (Glanzmann thrombasthenia). Patients with defects in platelet secretion and signal transduction are a heterogeneous group lumped together for convenience of classification rather than based on 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 large 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 the aggregation studies are nonspecific, and it is difficult to establish a specific 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 pro-coagulant–PS during platelet activation. Defects related to platelet cytoskeletal or structural proteins also may be associated with platelet dysfunction. Recent studies document impaired platelet function associated with mutations in transcription factors (eg, RUNX1, GATA1, FLI-1) that regulate the expression of important platelet proteins. In addition to these groups, some patients have abnormal platelet function associated with systemic disorders, such as Down syndrome and the May-Hegglin anomaly, in which the specific aberrant platelet mechanisms are unclear. The prevalence and relative frequencies of the various platelet abnormalities remain unknown.

Table 10-7 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)
2. Defects in platelet–platelet interaction (disorders of aggregation)
 - a. Congenital afibrinogenemia (deficiency of plasma fibrinogen)
 - b. Glanzmann thrombasthenia (deficiency or defect in GPIb-IIIa)
3. Disorders of platelet secretion and abnormalities of granules
 - a. Storage pool deficiency ($\delta\alpha\alpha\delta$)
 - b. Quebec platelet disorder
4. Disorders of platelet secretion and signal transduction
 - a. Defects in platelet–agonist interaction (receptor defects) (ADP, Thromboxane A2, Collagen, Epinephrine)
 - b. Defects in G-proteins (G α q, G α s, G α i Abnormalities)
 - c. Defects in phosphatidylinositol metabolism and protein phosphorylation
 - Phospholipase C- β 2 Deficiency
 - PKC- θ deficiency
 - d. Abnormalities in arachidonic acid pathways and thromboxane A₂ synthesis
 - Phospholipase A2 deficiency
 - Cyclooxygenase deficiency
 - Thromboxane synthase deficiency
5. Disorders of platelet coagulant–protein interaction (Scott syndrome)
6. Defects related to cytoskeletal/structural proteins
 - a. Wiskott-Aldrich syndrome
 - b. β 1-Tubulin deficiency
7. Abnormalities of transcription factors leading to functional defects
 - a. RUNX1 (familial platelet dysfunction with predisposition to acute myelogenous leukemia)
 - b. GATA1
 - c. FLI-1 (Dimorphic dysmorphic platelets with giant α -granules and thrombocytopenia; Paris-Trousseau/Jacobsen syndrome)

Modified with permission from Rao AK. Congenital disorders of platelet function: disorders of signal transduction and secretion.

Am J Med Sci. 1998;316:69-77.

FLI-1 = friend leukemia integration 1; GATA1 = sex-linked inheritance; GPIb = glycoprotein Ib; PKC = protein kinase C; RUNX1 = autosomal dominant; vWF = von Willebrand factor.

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 the higher 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 platelets, and these are reduced or abnormal in the BSS. Although GPV also is decreased in BSS platelets, it is not required for platelet surface GPIb-IX expression. The bleeding time is markedly prolonged, the platelet counts are moderately decreased, and on the peripheral smear, the platelets are markedly increased in size. In platelet aggregation studies, the responses to the commonly used agonists ADP, epinephrine, thrombin, and collagen are normal. Characteristically, the aggregation in platelet-rich plasma in response to ristocetin is decreased or absent, a feature shared with patients with vWD. This is because ristocetin-induced platelet clumping is mediated by binding of vWF to GPIb complex. Unlike in vWD, however, plasma vWF and factor VIII are normal in BSS, and the addition of exogenous vWF (present in plasma cryoprecipitate fractions) does not restore ristocetin-induced agglutination of platelets, because of the GPIb deficiency. Dense granule secretion on activation with thrombin may be decreased in these patients.

The blood film from a patient with BSS may resemble that from some patients with ITP in that the platelets tend to be larger than normal, and there is mild to moderate thrombocytopenia. The diagnosis of BSS is established by demonstrating decreased platelet surface GPIb, which can be performed using flow cytometry.

von Willebrand disease

See the section titled “von Willebrand disease” in Chapter 2.

Disorders of platelet aggregation

Glanzmann thrombasthenia

Glanzmann thrombasthenia is a rare autosomal recessive disorder characterized by markedly impaired platelet aggregation, a prolonged bleeding time, and relatively more severe mucocutaneous bleeding manifestations than most platelet function disorders. It has been reported in clusters in populations in which consanguinity is common. Normal resting platelets possess approximately 50,000-80,000 GPIIb-IIIa complexes on the 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. Because of this, fibrino-

gen binding to platelets on activation and aggregation are impaired. Clot retraction, a function of the interaction of GPIIb-IIIa with the platelet cytoskeleton, also is impaired.

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 virtually to all platelet agonists (except ristocetin), with the absence of both the primary and the 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 also is characterized by a similar absence of platelet aggregation, in this disorder, the prothrombin time, aPTT, and thrombin time are markedly prolonged, whereas they are normal in thrombasthenia. The diagnosis of thrombasthenia can be established by demonstrating decreased platelet expression of the GPIIb-GPIIIa complex using flow cytometry.

Disorders of platelet secretion and signal transduction

As a unifying theme, patients lumped in this remarkably heterogeneous group generally are characterized by impaired dense granule secretion and the absence of the 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 when the granule contents are diminished (storage pool deficiency [SPD]) or when the mechanisms mediating or potentiating aggregation and secretion are impaired (Table 10-7).

Deficiency of granule stores

SPD refers to patients with deficiencies in platelet content of dense granules (δ -SPD), α granules (α -SPD), or both types of granules ($\alpha\delta$ -SPD).

Patients with δ -SPD have a mild to moderate bleeding diathesis associated with a prolonged bleeding time. In the platelet studies, the second wave of aggregation in response to ADP and epinephrine is absent or blunted, and the collagen response is impaired markedly. Normal platelets possess 3-8 dense granules (each 200-300 nm in diameter). Under the electron microscope, dense granules are decreased in SPD platelets. By direct biochemical measurements, the total platelet and granule ATP and ADP contents are decreased

along with other dense granule constituents, 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, WAS, TAR syndrome, and Griscelli syndrome. The simultaneous occurrence of δ-SPD and defects in skin pigment granules, as in the HPS, point to the interrelatedness of the two kinds of granules (platelet and melanosome) with respect to genetic control.

In a large group of HPS patients in northwest Puerto Rico, HPS occurs in 1 of every 1,800 individuals. There are at least seven known HPS-causing genes, with most patients having HPS-1 and being from Puerto Rico. These HPS subtypes are autosomal recessive, and the heterozygotes have no clinical findings. In addition to the albinism, most patients have congenital nystagmus and decreased visual acuities. Two additional manifestations in HPS patients are granulomatous colitis and pulmonary fibrosis.

Chediak-Higashi syndrome is a rare autosomal recessive disorder characterized by SPD, oculocutaneous albinism, immune deficiency, cytotoxic T, and natural killer (NK) 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 have an isolated deficiency of platelet α-granule contents. The name refers to the initial observation that the platelets have a gray appearance with paucity of granules on the peripheral blood smears. These patients have a bleeding diathesis, mild thrombocytopenia, and a prolonged bleeding time. The inheritance pattern has been variable; autosomal recessive, autosomal dominant, and sex-linked patterns have been noted. 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 PDGF. 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 have been variable. Responses to ADP and epinephrine have been normal in most patients; in some patients, aggregation responses to thrombin, collagen, and ADP have been impaired.

The Quebec platelet disorder, another disorder affecting the platelet granules, 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. These patients are characterized by normal to

reduced platelet counts, proteolytic degradation of α-granule proteins, and defective aggregation selectively with epinephrine.

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 10-1). If the key components in signal transduction are the surface receptors, the G proteins, and the effector enzymes, evidence now exists for specific platelet abnormalities at each of these levels.

Patients with receptor defects have impaired responses because the platelet surface receptors for a specific agonist are decreased. Such defects have been documented for receptors for ADP, TXA₂, collagen, and epinephrine. Patients with the ADP receptor abnormalities have had a defect in the P2Y12 ADP receptor, which is coupled to inhibition of adenylyl cyclase and is the receptor targeted by thienopyridines (clopidogrel). Because ADP and TXA₂ play a synergistic role in platelet responses to several agonists, these patients with specific receptor defects manifest abnormal aggregation responses to multiple agonists. Patients described with abnormal platelet responses to collagen have had deficiencies in membrane GPs, GPIa, or GPVI.

G proteins are a link between surface receptors and intracellular effector enzymes, and defects in G protein activation can impair signal transduction. Patients with deficiencies at the level of Gαq, Gαi1, and Gαs have been described.

Patients have been described with impaired signal transduction resulting from defects in phospholipase C activation, calcium mobilization, and plectrin phosphorylation. Specific deficiencies at the level of phospholipase C-β2 and PKC-θ have been documented.

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 PS and phosphatidylethanolamine are located predominantly on the

inner leaflet, whereas phosphatidylcholine has the opposite distribution. Platelet activation results in a redistribution with expression of PS on the outer surface, mediated by phospholipid scramblase. The exposure of PS on the outer surface is an important event in the expression of platelet procoagulant activities. A few patients have been described in whom the platelet contribution to blood coagulation 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 have been normal.

Other abnormalities

Platelet function abnormalities have been described in association with other entities, such as in WAS, an X-linked inherited disorder affecting T-lymphocytes and platelets characterized by thrombocytopenia, immunodeficiency, and eczema. The bleeding manifestations are variable. Several platelet abnormalities, including dense granule deficiency and deficiencies of platelet GPIb, GPIIb-IIIa, and GPIa, have been reported in WAS. WAS arises from mutations in the gene coding for the WAS protein, which constitutes a link between the cytoskeleton and signaling pathways. Platelet dysfunction also occurs with mutations in tubulin-1, a cytoskeletal protein. More recently, abnormal platelet function has been documented in patients with mutations in transcription factors RUNX1 (also called core-binding factor A₂), GATA1, and FLI-1. Patients with RUNX1 mutations have familial thrombocytopenia, platelet dysfunction, and predisposition to acute leukemia.

Therapy 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 the 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 antibodies against GPIIb-IIIa and GPIb, respectively, which compromise the efficacy of subsequent platelet transfusions. An alternative to platelet transfusions is intravenous 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 have been variable, with a shortening of the bleeding time in some patients. More recently, recombinant factor

VIIa has been used in the management of bleeding events in patients with Glanzmann thrombasthenia and some other inherited defects. DDAVP and recombinant factor VIIa are not currently approved by the FDA for management of patients with inherited platelet defects; however, factor VIIa is approved in Europe to control bleeding in patients with thrombasthenia.

Key points

- Patients with inherited platelet defects typically have mucocutaneous bleeding manifestations; spontaneous hemarthrosis is rare.
- Patients with the BSS have thrombocytopenia, large platelet size, and a defect in 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 agonist 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 α-granule contents.
- In a substantial number of patients with abnormal aggregation responses, the underlying mechanisms are unknown. Some of the 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 etiologies (Table 10-8). The specific biochemical and pathophysiologic aberrations leading to platelet dysfunction are poorly understood in most of them. In some, such as the myeloproliferative neoplasms (MPN), 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 pharmacologic agents, artificial surfaces (cardiopulmonary bypass), compounds that accumulate in plasma due to impaired renal function, and antibodies. In these disorders of platelet dysfunction, the bleeding is usually mucocutaneous with a wide and unpredictable spectrum of severity. The usual laboratory tests that suggest a platelet dysfunction include a prolonged bleeding time and abnormal results in studies of platelet aggregation or the platelet function analyzer (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 the

Table 10-8 Disorders in which acquired defects in platelet function are recognized.

Uremia
Myeloproliferative disorders
Acute leukemias and myelodysplastic syndromes
Dysproteinemias
Cardiopulmonary bypass
Acquired storage pool deficiency
Acquired von Willebrand disease
Antiplatelet antibodies
Drugs and other agents

abnormalities in platelet aggregation studies and clinical bleeding remains weak.

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. The platelet abnormalities result from their development from an abnormal clone of stem cells, but some of the alterations may be secondary to enhanced platelet activation *in vivo*. The clinical impact of the *in vitro* qualitative platelet defects, which occur even in asymptomatic patients, often is unclear.

Numerous studies have examined platelet function and morphology in patients with MPN. Under the electron microscope, the platelet findings include reduction in dense and α -granules, alterations in the open canalicular and dense-tubular systems, and a reduction of 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 in response 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 idiopathic 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 MPN patients with elevated platelet counts and may contribute to the hemostatic defect. Plasma vWF, particularly the large vWF multimers, is decreased, is inversely related to the platelet counts, and has improved following cytoreduction. These changes in plasma vWF occur in patients with reactive thrombocytosis as well.

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 of these patients and have been attributed to coating of platelets 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 interaction, comorbid conditions, and the concomitant use of medications that affect hemostasis. The bleeding time may be prolonged; anemia also contributes to the prolongation, which may shorten following red blood cell transfusion or treatment with erythropoietin.

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. 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 dialysis ameliorates uremic bleeding diathesis in many patients. Hemodialysis and peritoneal dialysis are equally effective. Platelet transfusions are indicated in the management of acute major bleeds. Other treatments including DDAVP, cryoprecipitate, and conjugated estrogens also have been shown to be beneficial. Elevation of the hematocrit with packed red blood cells or recombinant erythropoietin may shorten bleeding times, 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 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 the 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 transplantation rejection, multiple congenital cavernous hemangioma, MPN, acute and chronic leukemias, and severe valvular disease, and in patients undergoing cardiopulmonary bypass.

Acquired von Willebrand disease

Acquired vWD (AvWD) is an often unrecognized bleeding disorder. Most patients are older (median age 62 years) without previous manifestations or a family history of a bleeding diathesis. The major associated disorders in these patients include lymphoproliferative disorder or plasma cell proliferative disorder, cardiac disease, MPN, and autoimmune disorders. Patients with MPN and reactive thrombocytosis demonstrate an impressive correlation between the plasma vWF abnormalities and elevated platelet counts. AvWD has been documented in patients with severe aortic stenosis and congenital valvular heart disease and in those with left-ventricular assist devices (LVAD), due to shear-stress induced loss of the high-molecular weight multimers of vWF from plasma. Laboratory findings for AvWD have included various combinations of a prolonged bleeding time, decreased plasma levels of vWF and factor VIII, and, most important, a selective reduction in the large vWF multimers in many of these disorders. The goals of treatment in AvWD are first to raise plasma vWF levels (DDAVP or vWF-containing factor VIII concentrates) to treat or prevent bleeding, and second to address the underlying associated conditions.

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. Patients with ITP have decreased platelet survival and some may have impaired platelet function and prolonged bleeding times even at adequate counts. In ITP patients, the antibodies are directed against specific platelet surface membrane GPs that play a major role in normal platelet function, including GPIb, GPIIb-IIIa, GPIa-IIa, and GPVI, and glycosphingolipids. Some of these antibodies may affect platelet function.

Drugs that inhibit platelet function

Many drugs affect platelet function. For several, the effects on platelets have been studied *in vitro*, and the relevance of such findings to the drug levels achieved in clinical practice is not well established. Even among those drugs shown to alter platelet responses *ex vivo*, the impact on hemostasis often remains unclear. Moreover, the impact of concomitant administration of multiple drugs, each with a mild effect on platelet function, is unknown, although this may be clinically relevant. 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 the inhibition of platelet aggregation and secretion upon stimulation with ADP, epinephrine, and low concentrations of collagen. Aspirin irreversibly acetylates and inactivates the platelet cyclooxygenase (COX-1), leading to the inhibition of synthesis of endoperoxides (prostaglandin G₂ and H₂) and TxA₂. Typically, it is recommended to wait 5–7 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. Several other nonsteroidal anti-inflammatory drugs also impair platelet function by inhibiting the cyclooxygenase enzyme and may prolong the bleeding time. Compared with aspirin, the inhibition of cyclooxygenase by these agents generally is short-lived and reversible (1–2 days). Cyclooxygenase-2 inhibitors do not inhibit platelet aggregation responses.

Ticlopidine, clopidogrel, and prasugrel are orally administered thienopyridine derivatives that inhibit platelet function by inhibiting the binding of ADP to the platelet P2Y12 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. GPIIb-IIIa receptor antagonists are compounds that inhibit platelet fibrinogen binding and platelet aggregation. These include a monoclonal antibody against the GPIIb-IIIa receptor (abciximab, ReoPro), a synthetic peptide

containing the RGD sequence (eptifibatide, Integrilin), and a peptidomimetic (tirofiban, Aggrastat). They are potent inhibitors of aggregation in response to all of the usual used agonists (except ristocetin) and prolong the bleeding time. DITP (secondary to drug-dependent antibodies) occurs in 0.2%-1.0% of patients on first exposure to GPIIb-III antagonists.

A host of other medications and agents, including oncologic drugs (eg, mithramycin) 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. These include carbenicillin, penicillin G, ticarcillin, ampicillin, nafcillin, azlocillin, cloxacillin, mezlocillin, oxacillin, piperacillin, and apalcillin. The platelet inhibition appears to be dose dependent, taking approximately 2-3 days to manifest and 3-10 days to abate after drug discontinuation. Cephalosporins also may impair platelet function. Moxalactam has been reported to induce platelet dysfunction associated with prolonged bleeding times and clinical hemorrhage. Other third-generation cephalosporins appear to show little effect on normal platelet function. The clinical significance of the effect of antibiotics on platelet function remains unclear. The general context in which the bleeding events are encountered in patients on antibiotics prevents identification of the precise role played by the antimicrobials because of the presence of concomitant factors (eg, thrombocytopenia, DIC, infection, vitamin K deficiency). Discontinuation of a specifically indicated antibiotic usually is not an option or necessary.

Evidence is growing that selective serotonin reuptake inhibitors (SSRIs) inhibit platelet function, and this has clinical relevance. Serotonin in plasma is taken up by platelets, incorporated into dense granules, and secreted on platelet activation. The SSRIs inhibit the uptake of serotonin and platelet aggregation and secretion responses to activation. In epidemiologic studies, patients on SSRIs have had increased gastrointestinal bleeding and increased bleeding with surgery. Last, 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.

Key points

- Alterations in platelet function are described in many disorders of diverse etiologies; the clinical significance in terms of relationship to bleeding manifestations remains unclear in many.
- 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 MPN may have altered platelet function that contributes to the bleeding manifestations.

Key points (continued)

- 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 the platelet dysfunction in these patients.

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Immune thrombocytopenia

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CHAPTER 11

Laboratory hematology

Charles S. Eby, John L. Frater, and Jacob H. Rand

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General concepts

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, heparin contamination, wrong collection tube or volume of blood, delayed processing). Additional causes of inaccurate laboratory results include sample mislabeling, analytical mistakes, and reporting errors.

Terminology

Sensitivity, specificity, and positive or negative predictive values are defined using the following clinical variables: true positive (TP; assay correctly identifies a condition 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*; [$TP/(TP + FP) \times 100$]) or the likelihood that a normal test indicates a patient without the abnormality (*negative predictive value*; [$TN/(TN + FN) \times 100$]). Positive and negative predictive values depend on the frequency of the abnormality being sought in the population as well as the sensitivity and specific of the laboratory methods.

Reference ranges are derived from a sample of a well population and typically reflect the results of 95% (mean ± 2 standard deviation [SD]) of disease-free individuals. The reference ranges of some assays are determined by the results of 98%–99% of disease-free individuals (mean ± 3 – 5 SD).

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Specific laboratory tests

Automated blood cell counting

In addition to complete blood counts (CBCs) and five-part leukocyte differential counts (LDCs), hematology analyzers

provide quantitative and qualitative information about reticulocytes, nucleated red blood cells (RBCs), and platelets. Additional information, such as platelet immaturity, extended leukocyte counts, and reticulocyte-specific indices, is available only from selected instrument manufacturers. Because of the large number of cells counted from each blood sample and analysis using multiple physical principles and sophisticated software, hematology analyzers generally produce accurate and precise CBCs and LDCs, 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. Hematology analyzers provide excellent sensitivity to distinguish between normal and abnormal samples via operator alerts prompting microscopic review of a stained peripheral blood smear for selected samples. As a result, approximately 30% of hospital patients' samples require review of a stained blood smear.

Automated blood cell counters use various technologies to enumerate and classify blood cells (Figure 11-1). Most platforms available for clinical use utilize at least two 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 electrical resistance as they flow through a narrow aperture and interrupt a direct electrical current. Software analysis defines RBCs, white blood cells (WBCs), 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.

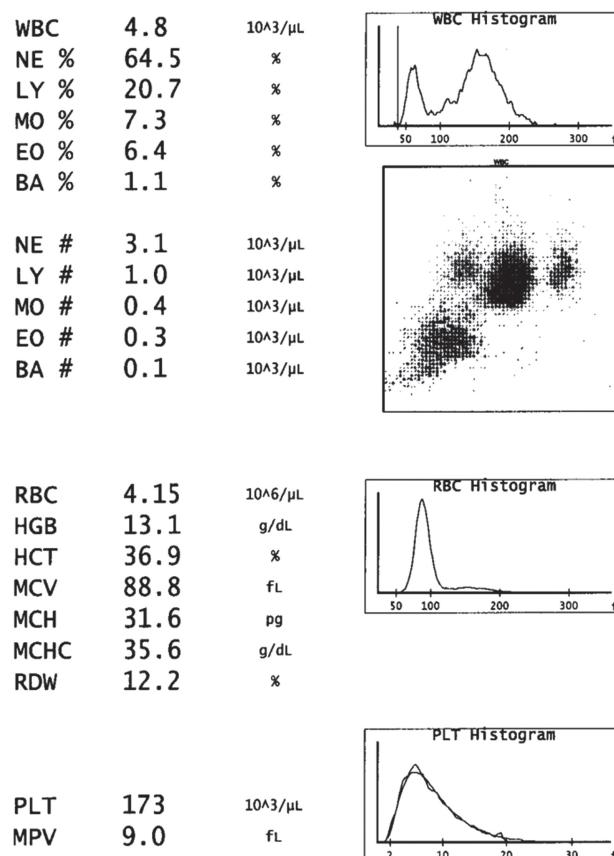


Figure 11-1 Data and histograms performed on a Beckman-Coulter LH 750 automated hematology analyzer from a healthy adult. The white blood cell (WBC), red blood cell (RBC), and platelet (PLT) histograms represent cell volumes determined by impedance. The second histogram from the top displays WBC light 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; HGB = hemoglobin; LY = lymphocyte; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MO = monocyte; MPV = mean platelet volume; NE = neutrophil; RDW = red blood cell distribution width.

The amount of light scattered at a wide angle depends on such factors as cytoplasmic granules and nuclear shape. All of the major hematology analyzer manufacturers use light-scattering technology.

Fluorescence

In addition to the physical properties of cells, fluorochrome-labeled antibodies recognizing cell surface or intracellular epitopes 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), nucleated RBCs (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 $10^6/\text{mL}$) and volume (mean corpuscular volume [MCV], reported in units of 10^{-15} L) of RBCs, and hemoglobin concentration (reported in units of g/dL) after lysing red blood cells; 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 measurement can be elevated artifactually by increased sample turbidity because of leukocytosis, paraproteinemia, carboxyhemoglobinemia, hyperbilirubinemia, or hyperlipidemia.

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 one 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 (10^{-15} L) \times red blood cells ($3 \cdot 10^{12}/\text{L}$) \times 100.

The mean corpuscular hemoglobin (MCH) is expressed in picograms (10^{-12} g). The MCH is calculated by dividing hemoglobin (g/L) by red blood cell count ($10^{12}/\text{L}$). 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.

The red blood cell 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, and also is elevated more frequently with macrocytic anemias due to vitamin B12 or folate deficiency than to liver disease, hypothyroidism, or a reticulocytosis. Myelodysplastic syndromes, such as refractory anemia, or RBC transfusions to 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 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 others. 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.

Nucleated red blood cells

Circulating nucleated red blood cells (NRBCs) occur in newborns; however, beyond this period, the presence of NRBCs is abnormal and is associated with various hematopoietic stresses, including hemolytic anemias, myeloproliferative disorders, metastatic cancer to bone marrow, and hypoxia. All major hematology analyzer brands enumerate NRBCs and correct WBC and lymphocyte counts for interference from NRBCs analyzer.

Leukocyte analysis

To differentiate lymphocytes, monocytes, neutrophils, eosinophils, and basophils, most instruments use impedance and/or light scattering, plus additional physical properties. Coulter and Sysmex use radiofrequency conductivity, and Advia (Siemens) uses peroxidase staining. 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, including metamyelocytes, myelocytes, promyelocytes, and blasts. Some Sysmex analyzers identify a subset of WBCs called hematopoietic progenitor cells, which correlate with CD34 counts and can be used to monitor peripheral stem cell mobilization.

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), however, leading to inaccurate MPV results and thus diminishing its clinical utility. Inaccurate automated platelet counts can result from fragmented RBCs, congenital (inherited macrothrombocytopenia disorders such as May-Hegglin anomaly) or acquired (myeloproliferative disorders or idiopathic thrombocytopenic purpura) 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 contain detectable mRNA. Currently, only certain analyzers provide an immature platelet fraction (IPF), with a reference range of 1.1%–6.1%, 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 stained with either the Wright or the May-Grunwald-Giemsa stains and can be prepared by automated slide maker or strainers, which can be interfaced with hematology analyzers. Microscopic examination of stained blood smears can identify morphologic abnormalities that automated hematology analyzers nonspecifically flag or, rarely, miss. The 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 11-1), where there are only a few overlapping RBCs and central pallor of normal red blood cells 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 CellaVision 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, automated 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.

Supravital stains are used to detect RBC inclusions. 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 cells in α -thalassemias).

Bone marrow aspirate and biopsy

Following are the most frequent indications for bone marrow biopsy: 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; and unexplained splenomegaly. Bone marrow aspirate and biopsy commonly are performed by collecting specimens from the posterior iliac crests. 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 prep are stained with either the Wright or May-Grunwald-Giemsa stains; unstained smears should be retained and kept frozen for possible special stains. The aspirate is used for cytologic examination of the bone marrow cells and for performing the differential. Bone marrow core biopsies are fixed in formalin, and the biopsy specimen is decalcified and embedded in paraffin; 3–4 mm sections are then cut and stained with hematoxylin and eosin or Giemsa stains.

When examining pediatric marrows, it is understood that erythroid hyperplasia is present at birth because of high levels of erythropoietin. Lymphocytes may compose 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 nucleated red blood cells (sideroblastic iron) and histiocytes (reticuloendothelial iron). See Table 11-2 for other cytochemical stains.

Ringed sideroblasts are abnormal nucleated red cells with blue-staining iron granules surrounding at least two-thirds of the nucleus. These iron granules are present in mitochondria

Table 11-1 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	Punctate basophilic inclusions	Precipitated ribosomes	Lead toxicity, thalassemias
Bite cells (degmacyte)	Smooth semicircle taken from one 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, uremia, bleeding ulcers, gastric carcinoma
Cabot ring	Circular, blue, threadlike inclusion with dots	Nuclear remnant	Postsplenectomy, hemolytic anemia, megaloblastic anemia
Howell-Jolly bodies	Small, discrete basophilic dense inclusions; usually single	Nuclear remnant	Postsplenectomy, hemolytic anemia, megaloblastic anemia
Pappenheimer bodies	Small dense basophilic granules	Iron-containing siderosomes or mitochondrial remnant	Sideroblastic anemia, postsplenectomy
Schistocytes (helmet cells)	Distorted, fragmented cell, with 2-3 pointed ends	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, immunohemolytic anemia
Stomatocytes	Mouth- or cuplike deformity	Membrane defect with abnormal cation permeability	Hereditary stomatocytosis, immunohemolytic anemia
Target cell (codocyte)	Targetlike appearance, often hypochromic	Increased redundancy of cell membrane	Liver disease, postsplenectomy, thalassemia, HbC
Teardrop cell (dacrocyte)	Distorted, drop-shaped cell		Myelofibrosis, myelophthisic anemia

*Blood smear abnormalities can be artifacts of poor slide preparation or viewing the wrong part of the smear.

G6PD = glucose-6-phosphate dehydrogenase; HbC = hemoglobin C.

Modified from Kjedsberg C, ed. *Practical Diagnosis of Hematologic Disorders*. 2nd ed. Chicago, IL: ASCP Press; 1995.

Table 11-2 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.
Tryptase	Positive in mast cells, negative in basophils. Mast cells in systemic mast cell disease frequently have a spindled shape.

surrounding the nuclear membrane. Iron staining of the biopsy can underestimate the marrow iron stores because of the loss of iron during decalcification.

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 blood smears, marrow aspirates, and bone marrow biopsies or other tissues. Many cytochemical stains, such as tartrate-resistant acid phosphatase (TRAP) and myeloperoxidase, have been converted into immunohistochemical (IHC) reactions.

IHC stains are used on marrow aspirates and blood smears as an alternative or adjunct to flow cytometry. The advantage of immunohistochemistry 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), immunohistochemistry 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 a sterile tube containing tissue culture medium such as RPMI (containing fetal bovine serum, L-glutamine, and antibiotics) and anticoagulant.

Paraffin-embedded tissue can be used for polymerase chain reaction (PCR) of genomic DNA sequences. Reverse transcriptase PCR (RT-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 cytoplasmic proteins using fluorescent-labeled monoclonal antibodies or the assessment of DNA content using DNA-binding dyes.

Flow cytometry is used for phenotyping populations of cells, enumerating early progenitors for stem cell transplants, detecting minimal residual disease, detecting targets for immunotherapy, and assessing the presence of prognostic markers. See Table 11-3 for a summary of clinical uses of flow cytometry in 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,

Table 11-3 Specimen allocation for ancillary studies.

Clinical problem	Ancillary techniques
Pancytopenia	Flow cytometry (LGL, hairy cell leukemia, PNH clone, AML) Cytogenetics (AML, MDS) Molecular genetics
Myeloid leukemia	Flow cytometry (phenotyping) Cytogenetics and FISH Molecular genetics (<i>BCR-ABL</i> , <i>PML/RARA</i> , <i>AML1/ETO</i>)
Lymphoproliferative disorder	Flow cytometry (phenotyping, prognostic markers such as ZAP-70) Cytogenetics: t(1;19) in pre-B-cell ALL, t(14;18) in follicular lymphomas, etc. FISH Molecular genetics (clonality, specific markers such as <i>BCL2</i> , <i>BCL6</i> , etc.) Immunohistochemistry (phenotyping, prognostic markers)
Myeloproliferative disorders	Cytogenetics FISH (<i>BCR-ABL</i>) Molecular genetics (<i>BCR-ABL</i> , <i>JAK2</i>)
Plasmacyt proliferative disorders	Flow cytometry (phenotyping, labeling index) Cytogenetics

ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CLL = chronic lymphocytic leukemia; FISH = fluorescence in situ hybridization; LGL = large granular lymphocyte leukemia; MDS = myelodysplastic syndrome; PNH = paroxysmal nocturnal hemoglobinuria.

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 DNA, a feature of CLL arising from pre-germinal center cells, and patients with pre-germinal center CLL have a decreased overall survival when compared with patients with CLL arising from post-germinal center cells. Positivity for CD38 by flow cytometric analysis also is correlated with unmutated DNA, but the correlation is not as strong as it is with ZAP-70.

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 unit—granulocyte, macrophage (CFU-GM, CD34+, 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.

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 one 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 11-4 through 11-10 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 two 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, respectively. 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 red cells or Rh⁺ and Rh⁻ red cells during pregnancy and postpartum and can identify red cell 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.

Table 11-4 Clinically useful CD markers.

Marker	Lineage association
<i>Progenitor cells</i>	
CD34	Progenitor cells, endothelium
CD38	Myeloid progenitors, T, B, NK cells, plasma cells, monocytes, CLL subset
<i>B-cell markers</i>	
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)
CD 103	Intraepithelial lymphocytes, hairy cell leukemia, T-cells in enteropathic T-cell lymphoma
FMC7	B-cells (not typical CLL), hairy cell leukemia
<i>T-cell markers</i>	
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
<i>NK/cytotoxic T-cell markers</i>	
CD16	NK cells, monocytes, macrophages, neutrophils
CD56	NK cells, myeloma cells
CD57	NK cells, T-cell subset
<i>Myeloid and monocytic markers</i>	
CD13	Monocytes, neutrophils, eosinophils, and basophils
CD14	Monocytes, macrophages, subset of granulocytes
CD33	Myeloid lineage cells and monocytes
CD117	Immature myeloid cells, AML
<i>Monocytes</i>	
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
<i>Megakaryocytic markers</i>	
CD41	Platelets and megakaryocytes (GPIIb)
CD42	Platelets and megakaryocytes (CD42a: GPI; CD42b: GPIb)
CD61	Platelets, megakaryocytes, endothelial cells (GPIIb-IIIa)
<i>Erythroid markers</i>	
CD71	Transferrin receptor is upregulated upon cell activation
CD235a	Glycophorin A

AML = acute myelogenous leukemia; B-ALL = B-lineage acute lymphoblastic leukemia; CLL = chronic lymphocytic leukemia;
 GP = glycoprotein; NK = natural killer.

Table 11-5 Acute myeloid leukemia phenotyping.

	HLA-DR	CD34	CD33	CD13	CD11c	CD14	CD41	CD235a
M0	+	+	+	+/-	+/-	-	-	-
M1	+	+	+	+	+/-	+/-	-	-
M2	+/-	+/-	+	+	+/-	+/-	-	-
M3	-	-	+	+	+/-	-	-	-
M4	+	+/-	+	+	+	+	-	-
M5	+	-	+	+	+	+	-	-
M6	+/-	-	-	-	+/-	-	-	+
M7	+/-	+/-	+/-	-	-	-	+	+

Table 11-6 B-lineage acute lymphoblastic leukemia phenotyping.

	TDT	CD19	CD10	CD20	Cyto-m	Surface Ig
Pro-B	+	+	-	-	-	-
Pre-Pre-B (common ALL)	+	+	+	-	-	-
Pre-B	+	+	+	1/-	+	-
Early B (Burkitt)	-	+	+	+	-	+

Cyto-m = cytoplasmic m; Ig = immunoglobulin; TDT = terminal deoxynucleotidyl transferase.

Table 11-7 T-lineage acute lymphoblastic leukemia phenotyping.

Surface	TDT	CD7	CD2	CD5	CD1	CD3	CyCD3	CD4/CD8
Prothymocyte	+	+	+	-	-	-	+	-/-
Immature thymocyte	+	+	+	+	-	-	+	-/-
Common thymocyte	+	+	+	+	+	+/-	+	+/-
Mature thymocyte	-	+	+	+	-	+	+	CD4 or CD8 ¹
Mature T cell	-	+	+	+	-	+	+	CD4 or CD8 ¹

Cy CD3 = cytoplasmic CD3; TDT = terminal deoxynucleotidyl transferase.

Table 11-8 Common B-cell neoplasms.

	CD20	CD5	CD10	CD23	CD43	C Ig	S Ig	Cyclin D1	Other
CLL/SLL	+	++	-	++	++	5%+	+	-	FMC7 ⁻ , CD79b ⁺
LPL	++	-	-	-	+/-	+	+	-	
PLL	++	+/-		-			++	-	
HCL	++	-	-	-	-	-	+	+/-	CD11c ⁺ , CD25 ⁺ , CD103 ⁺
MCL	++	++	-	-	++	-	++	++	FMC7 ⁺
MZL	++	-	-	-	+/-	+/-	++	-	
FCL	++	-	60%+	-	-	-	++	-	BCL2 ⁺
LCL	++	10%+	25%-50%+	+	+/-	+/-	+/-	-	BCL2 ⁺ in 30%-40%
Burkitt	++	-	+	-	-	+	+	-	
Myeloma	-	-	Occ +	-	+	++	-	15%-20%+	CD56 ⁺ , CD38 ⁺ , CD138 ⁺

C Ig = cytoplasmic immunoglobulin; CLL = chronic lymphocytic leukemia; FCL = follicular center cell lymphoma; HCL = hairy cell leukemia; LCL = large-cell lymphoma; LPL = lymphoplasmacytic lymphoma; MCL = mantle cell lymphoma; MZL = marginal zone lymphoma; PLL = B-cell prolymphocytic leukemia; S Ig = surface immunoglobulin; SLL = small lymphocytic lymphoma.

Table 11-9 Common mature T-cell and NK-cell neoplasms.

	CD3S	CD3C	CD5	CD7	CD4	CD8	CD30	CD16	CD56	EBV
T-PLL	+	+	-	+	4.8	4.8	-	-	+	-
T-LGL	+	+	-	+	-	+	-	+	-	-
NK-leukemia	-	-	-	+/-	-	+/-	-	-	+	+
EN-NK/T	-	+	-	+/-	-	-	-	+	+	+
HS-gd lym	+	+	-	+	-	-	-	+	+/-	-
Ent-T lym	+	+	+	+	-	+/-	+/-	-	-	-
SC pannic T lym	+	+	+	+	-	+	+/-	-	-	-
PTCL-NOS	+	-	+/-	+/-	+/-	+/-	+/-	-	+	+/-
AILD	+	+	+	+	+/-	-	-	-	+	+/-
ALCL	+	-	+/-	+/-	+/-	+/-	+	-	-	-

AILD = angioimmunoblastic lymphadenopathy; ALCL = anaplastic large-cell lymphoma; CD3C = cytoplasmic CD3; CD3S = surface CD3; EBV = Epstein-Barr virus; Ent-T lym = enteropathic T-cell lymphoma; EN-NK/T = extranodal natural killer/T-cell lymphoma; HS-gd lym = hepatosplenic gamma delta lymphoma; NK-leukemia = natural killer cell leukemia; PTCL-NOS = peripheral T-cell lymphoma, not otherwise specified; SC pannic T lym = subcutaneous panniculitis T-cell lymphoma; T-LGL = T-large granular lymphocyte leukemia; T-PLL = T-prolymphocytic leukemia.

Table 11-10 Immunohistochemical diagnosis of Hodgkin disease.

	CD45	CD30	CD15	CD20	CD3	Pax5
Hodgkin (R-S cells)	-	+	+	LPHD(+)	-	weak+
B-lymphoma	+	+/-	-	+	-	+
T-lymphoma	+	+/-	+/-	-	+	-

LPHD = lymphocyte-predominant Hodgkin disease; R-S = Reed-Sternberg.

Cytogenetically, a minimum of two mitotic cells with gain of the same chromosome or with the same structural abnormality or three mitotic cells with loss of the same chromosome define a clone.

Constitutional chromosome abnormalities, associated with either congenital genetic syndromes or normal variants, are determined on peripheral blood T-lymphocytes grown in culture with phytohemagglutinin (PHA), a T-cell mitogen.

Fluorescence in situ hybridization (FISH) is a cytogenetic technique that uses specific fluorescently 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.

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.

Molecular diagnostics

Polymerase chain reaction

PCR is designed to permit selective amplification of a specific target DNA sequence within total genomic DNA or a complex complementary DNA (cDNA) 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 four 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-ABL*)

translocation in chronic myeloid leukemia, detection of pro-myelocytic leukemia-retinoic acid receptor alpha (*PML-RARA*) in acute promyelocytic leukemia, and detection of the Janus kinase-2 (*JAK2*) mutation in polycythemia vera. Essential and primary myelofibrosis PCR is of increasing importance in the diagnosis of acute myeloid leukemia, particularly for the detection of internal tandem duplications in the FMS-like tyrosine kinase 3 (*FLT-3*) locus, mutations in CCAAT/enhancer binding protein α CCAAT enhancer binding protein alpha (*CEBPA*), and point mutations in the nucleophosmin 1 (*NPM1*) and Wilms tumor 1 (*WT1*) genes.

DNA sequencing is important in the identification of point mutations. The earlier Sanger (chain termination) technique rapidly is being eclipsed by the next-generation sequencing technology, which has a high throughput capacity and thus makes the parallel analysis of many genes possible. The potential clinical uses, including diagnosis, predictors of response to therapy, and risk stratification are being explored for a variety of malignancies, including myeloma, leukemias, and lymphoma.

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 immunoglobins, neutralize red blood cell 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 dyscrasia-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 devices 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 (Hgb S) rely on visual detection of turbidity when blood containing Hgb S is added to a solution containing a reducing agent, detergent to lyse red blood cells, and high-concentration salt buffer. Deoxygenated Hgb 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 Hgb S trait, Hgb S homozygous, and Hgb S β -thalassemia. 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 Hgb S concentration is <2.6 g/dL. Because the concentration of Hgb 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 Hgb S and to identify coexisting non-sickling hemoglobinopathies or thalassemias. Other rare hemoglobinopathies produce a positive solubility test, including hemoglobin C Harlem, and if coinherited with Hgb S, they will produce a sickle cell disease phenotype.

Hemoglobin electrophoresis

Methods to separate normal (hemoglobins 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 11-2). 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 Hgb S). HPLC and capillary electrophoresis instruments 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 and other uncommon hemoglobinopathies and β -thalassemia trait (elevated Hgb 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

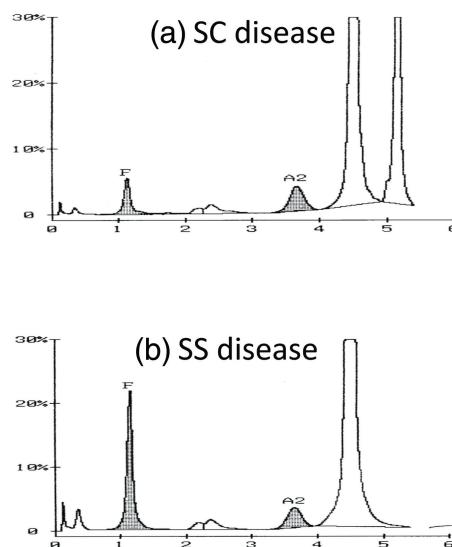
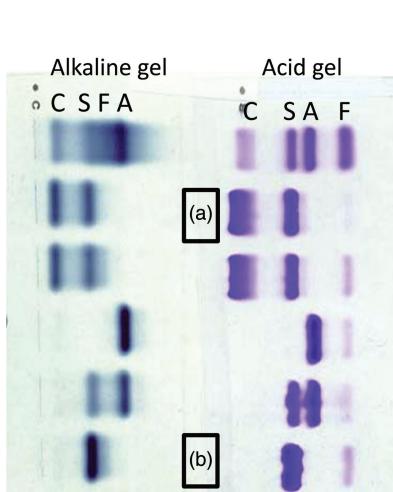


Figure 11-2 Examples of alkaline and acid gel electrophoresis and high-performance liquid chromatography patterns for (a) a patient with hemoglobin SC disease and (b) a patient with homozygous SS sickle cell disease.

acute illness, medications, or, rarely, ingestion of fava beans. Decreased G6PD activity diminishes nicotinamide adenine dinucleotide phosphate (NADPH) production 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, however, 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 a panel of additional RBC enzyme tests.

Hereditary red cell skeletal disorders

The unique flexibility of a red cell 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 red cell cytoskeleton defects are an infrequent cause of nonimmune 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 7 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 (DAT), anemia with reticulocytosis, mild splenomegaly, and spherocytes on blood smear (typically <10% of red cells). 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 red cell membrane, anchoring proteins, and 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 red cell membrane proteins. Hereditary elliptocytosis causes minimal, if any, anemia and is a morphologic diagnosis (normal OF and EMA binding). Pyropoikilocytosis is caused by inheritance of both qualitative and quantitative red cell skeletal defects, which produce severe hemolytic anemia, deranged red cell morphologies, and decreased EMA.

Hemostasis and thrombosis

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 of patients with underlying disorders associated with bleeding complications, evaluation of patients with personal or family histories of abnormal bleeding or bruising, assessment for inherited and acquired thrombosis risk factors, and antithrombotic drug monitoring.

Hemostasis screening typically consists of a prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet count. Abnormal screening test results require additional clinical and laboratory investigation to determine the etiologies. Mucosal bleeding, menorrhagia, petechiae, and ecchymoses suggest primary hemostasis disorders such as von Willebrand disease (vWD) and qualitative platelet disorders, whereas hematomas, hemarthroses, and delayed bleeding suggest a coagulation defect.

Testing for thrombophilia usually is 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 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. The levels of protein C (PC), protein S (PS), and antithrombin can decrease during the acute phase of a VTE. PC and PS levels are reduced by anticoagulation with warfarin. Antithrombin levels are lower during anticoagulation with unfractionated heparin. Lupus anticoagulant (LAC) testing ideally should be done before anticoagulation is begun, in conjunction with serologic assays (anticardiolipin and β -2-GPI antibodies), and abnormal results should be repeated at least 12 weeks later to determine whether they are persistent to fulfill the laboratory criteria for antiphospholipid antibody syndrome (APS). Genetic thrombophilia testing (factor V Leiden and prothrombin gene mutation

20210) can be ordered at any time and is not affected by clinical status or medications. Heparin-induced thrombocytopenia (HIT) and thrombotic thrombocytopenia (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 largely should be based on clinical assessment.

Two major forms of anticoagulation therapy, warfarin antagonism of vitamin K-dependent γ -carboxylation of coagulation factors X, IX, VII, and II, and unfractionated heparin, require therapeutic drug monitoring because of unpredictable anticoagulant activities. Efforts to harmonize 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.

It is important to be aware of the differences in hemostasis factor reference ranges among infants, older children, and adults to avoid mislabeling newborns with inherited defects (see Chapter 2 for details). The following sections provide specific information regarding hemostasis laboratory methods as they apply to the aforementioned clinical situations.

Screening coagulation testing

Most coagulation reactions are believed to be initiated by tissue factor activation of factor VII. Important interactions occur between the extrinsic and intrinsic pathways. Although the division into two separate pathways, as shown in Figure 11-3, does not reflect complex interactions among coagulation

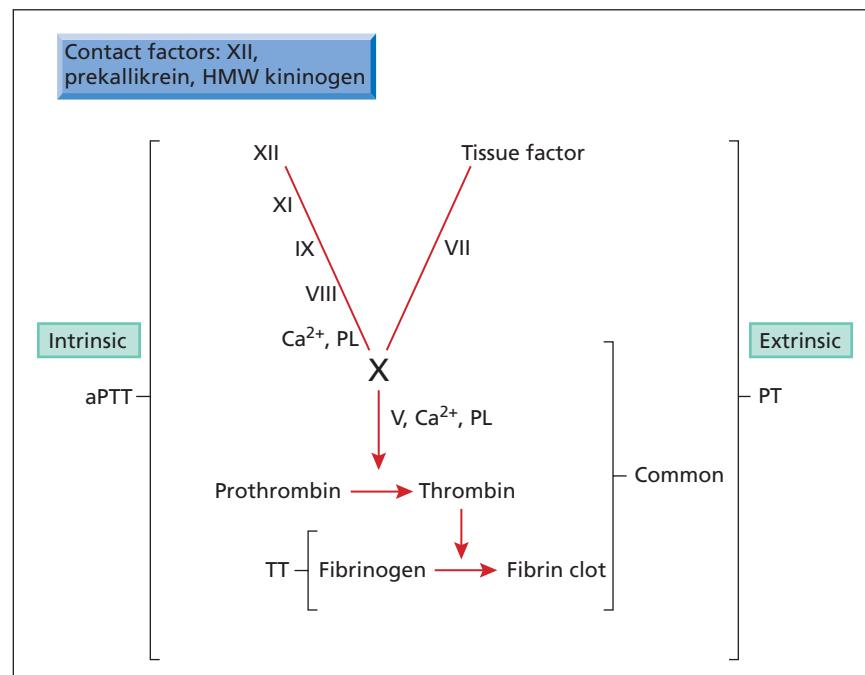


Figure 11-3 Simplified coagulation cascade indicating the intrinsic pathway measured by the activated partial thromboplastin time (aPTT), the extrinsic pathway measured by the prothrombin time (PT), the common pathway (factor X, factor V, prothrombin, and fibrinogen) measured by PT and aPTT, and the conversion of fibrinogen to fibrin measured by the thrombin time (TT). HMW = high molecular weight.

factors, it does provide a useful way to interpret screening coagulation test results when evaluating a clinical problem.

Preanalytical variables

The aPTT and, to a lesser degree, the PT are sensitive to changes in the ratio of sodium citrate solution in the collection tube and added plasma. Filling a tube with less than the recommended volume to obtain a sodium citrate-to-blood ratio of 1:9 or collecting blood in the proper 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 to artifactual prolongation of the aPTT. Heparin contamination due to blood collection from central lines can cause a prolonged aPTT. A prolonged thrombin time (TT) that corrects when repeated after treatment of plasma with a heparin-neutralizing confirms heparin contamination. Alternatively, a prolonged TT and a normal Reptilase time, which uses a snake venom not neutralized by heparin-accelerated anti-thrombin, will confirm the presence of heparin. Most PT

reagents contain heparin-neutralizing agents, making this screening clotting test insensitive to heparin contamination. Many coagulation tests performed on plasma from patients taking oral direct thrombin (dabigatran) and factor Xa (rivaroxaban) anticoagulants are at risk for either positive or negative biases, which can be clinically important (Table 11-11). 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 one or more coagulation factors or to an inhibitory antibody. The first step is to rule out contamination with heparin or direct thrombin inhibitors by performing a TT; if the TT is prolonged, the cause must be determined before proceeding. Next, the aPTT or PT is repeated on a 1:1 mixture of patient plasma and pooled normal plasma (PNP), which should provide at least 50% activity for all coagulation factors and substantial correction if deficiency is the cause. 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 1-2 hours followed by repeating the aPTT. There is no consensus approach for interpretation of

Table 11-11 Coagulation tests interference caused by new direct oral anticoagulants.

Test	Dabigatran oral factor IIa inhibitor	Rivaroxaban and other oral factor Xa inhibitors	Comments
APCr ratio	+bias	+bias	Risk of missing FVL
Antithrombin, anti-Xa method		+bias	Risk of missing AT deficiency
Antithrombin, anti-IIa Method	+bias		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
LA screen	prolonged	prolonged	
LA screen/confirm	prolonged	prolonged	Risk of false + LA
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 based on clot formation	-bias with some methods	unaffected	
Chromogenic anti-Xa	unaffected	positive bias	Not a quantitative test for Rivaroxaban unless calibrated with the drug
Monitoring of heparin/LMWH			

aPTT = activated partial thromboplastin time; AT = antithrombin; HMWK = high-molecular weight kininogen; LA = lupus anticoagulant; LMWH = low-molecular weight heparin; PC = protein C; PK = prekallikrein; PS = protein S.

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 (bleeding or thrombosis events) and the initial extent of PT and aPTT prolongation when assessing the 1:1 mix results. Sometimes mixing studies will not be definitive, especially when an aPTT is mildly prolonged and corrects with mixing, in which case performing both selected factor activity assays and LAC screening will be necessary.

Coagulation factor activity assays

Determination of a coagulation factor activity in a patient's plasma typically is performed on automated instruments and requires two reagents: PNP and plasma completely 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. PTs are performed on the calibration samples 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 on 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. To determine whether the inhibitor interference is specific for one factor, such as factor VIII, or nonspecific like an LAC may require performance of additional factor activities.

The end point for most coagulation tests is detection of a fibrin clot. A factor VIII chromogenic activity assay exists but is not widely used. The end point of this assay 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 than clotting assays, but they may not detect some defects in a factor that disrupt the binding of the factor to its natural (larger) substrate.

Prothrombin time

The PT measures the time to form a fibrin clot after adding thromboplastin (tissue factor combined with phospholipid) and CaCl_2 to citrated plasma and assesses three of the four

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. Congenital deficiencies of factors X, V, and II and fibrinogen are rare (1 in 1-2 million people), whereas the estimated prevalence of homozygous factor VII deficiency 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 autoantibodies are extremely rare (Figure 11-4).

Warfarin causes prolonged PTs (and variably, prolonged aPTTs 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),

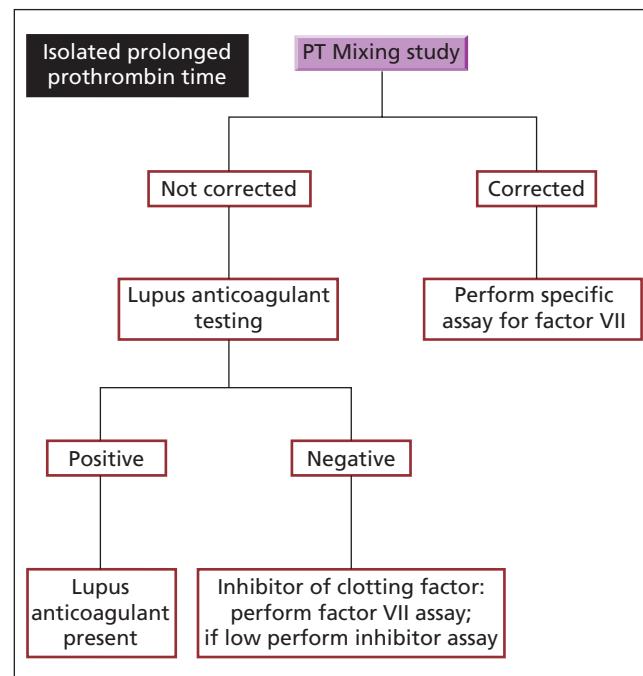


Figure 11-4 Algorithm for evaluation of an isolated prolonged prothrombin time.

manufacturers compare PTs obtained with commercial thromboplastin lots to a World Health Organization reference thromboplastin, with the behavior of 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 mean normal PT raised to the exponent of the thromboplastin ISI.

The reference range for an INR is typically 1.0 ± 0.2 . The INR is designed to accurately monitor patients who have been stabilized on warfarin. It is *not* intended for assessing coagulopathies due to liver disease or new direct factor IIa and factor Xa inhibitors because the ISI is not validated for these conditions.

Activated partial thromboplastin time

The aPTT is a two-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 the 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 artifacts, congenital factor deficiencies, acquired inhibitors, and anticoagulation therapies (Figure 11-5).

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 instrument in use, for an isolated intrinsic factor deficiency to prolong the aPTT, activity is usually <30%-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%-40%).

Patients with type 1 vWD may have a slightly prolonged aPTT if the factor VIII level is low. Patients with the Normandy type 2 variant of vWD can have a moderate factor VIII deficiency.

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.

LACs can cause a prolonged aPTT (see section on thrombophilia testing). If a prolonged aPTT does not substantially

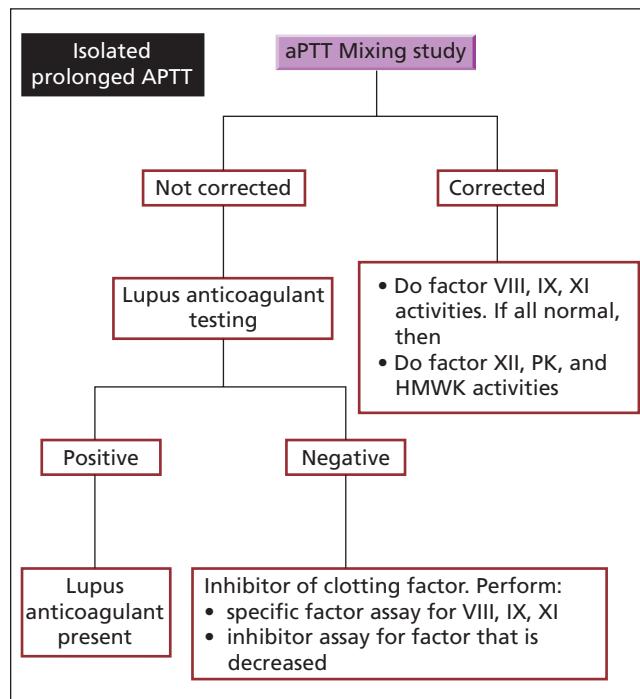


Figure 11-5 Algorithm for evaluation of an isolated prolonged activated partial thromboplastin time (aPTT). HMWK = high-molecular weight kininogen; PK = prekallikrein.

shorten when repeated on a 1:1 mix with PNP, perform LAC testing or specific factor activities, depending on the clinical context.

Inhibitors to factor VIII are detected in 25%-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 patient with 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 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. A BU of 0.5-5.0 is a low titer and may be overwhelmed with larger infusions of factor VIII, whereas a BU >10 will require treatment of bleeding episodes with a factor VIII bypass product, such as

recombinant factor V11a. The Nijmegen modification of the 1:1 mix conditions improves accuracy and precision of the Bethesda assay for low-titer inhibitors.

Most hospitals use aPTT-based nomograms to guide therapeutic anticoagulation with unfractionated heparin. A therapeutic aPTT range typically is determined by collecting plasma samples from patients on heparin infusions and comparing aPTTs to heparin activity using the anti-Xa chromogenic assay. The aPTT therapeutic range in seconds will correspond to an anti-Xa range of 0.3–0.7 IU/mL. The aPTT also is used to monitor the parenteral direct thrombin inhibitor argatroban, and the therapeutic target recommended by the manufacturer 1.5–3.0 times the baseline aPTT. Therapeutic infusions of direct thrombin inhibitors also prolong the PT or INR, and the intensity depends on the specific direct thrombin inhibitor and the thromboplastin reagent. The new generation of oral direct thrombin and anti-Xa inhibitors prolong the aPTT and PT (Table 11-11). They cannot be used to predict plasma concentrations, however. At the time of writing, quantitative assays to measure drug levels are not widely available.

The anti-Xa assay is a variation of a chromogenic anti-thrombin assay (see section on thrombophilia testing) comparing an unknown concentration of heparin in the patient plasma to a calibration curve prepared with an unfractionated heparin standard. Activated factor Xa is added to the test plasma, the rate of factor Xa neutralization by antithrombin is positively correlated with the heparin concentration, and the rate of chromogenic substrate cleavage by factor Xa is correlated inversely with the heparin concentration. Directly monitoring heparin anticoagulation with the anti-Xa assay is the preferred approach in some hospitals and is an alternative to the aPTT when unusually high rates of heparin infusion

are required or when a patient's baseline aPTT is prolonged because of an LAC or deficiency of a contact activator (factor XII, PK, or HMWK). Low-molecular weight heparins (LMWHs) will minimally prolong the aPTT at therapeutic concentrations. LMWHs typically do not require monitoring. Under certain situations, however, including patients of extremely low and high weights, pregnant patients, and patients with impaired renal function, monitoring plasma LMWH activity approximately 4 hours after a subcutaneous injection using a chromogenic anti-Xa assay calibrated against an LMWH is recommended.

Combined abnormalities of PT and aPTT

Deficiency or inhibition of a factor in the common pathway (factors X, V, II, and fibrinogen), hypofibrinogenemia, dysfibrinogenemia disseminated intravascular coagulation (DIC), and lupus can cause combined prolongation of the PT and aPTT. Advanced liver disease can cause decreased hepatic synthesis of all coagulation factors, except for factor VIII, and acquired dysfibrinogenemia, which is suggested by a low fibrinogen level in a functional assay combined with a normal or high level of immunologic fibrinogen (see the section on fibrinogen assays). See Figure 11-6 for evaluation of a prolonged PT and aPTT.

Symptomatic inhibitors to factor V rarely occur after patient exposure to bovine thrombin (which also contains bovine factor V) is 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. Low-factor V activity and specific in vitro inhibition of factor V confirm the diagnosis. Fortunately,

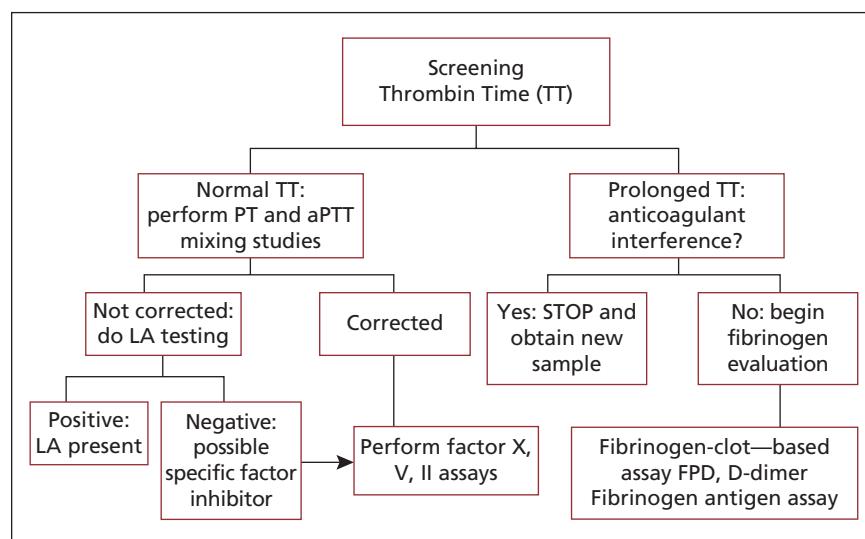


Figure 11-6 Algorithm for evaluation of a prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT). FDP = fibrin degradation product.

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.

Thrombin time

The TT measures the time required to convert fibrinogen to a fibrin clot, bypassing the intrinsic, extrinsic, and common pathways. TT requires sufficient amounts of normal fibrinogen and an absence of thrombin inhibitors and substances that impede fibrin polymerization. The only reagent is bovine or human thrombin, and the test sample is undiluted citrated plasma.

- Unfractionated heparin, LMWH, argatroban, bivalirudin, and dabigatran inhibit thrombin and prolong the TT.
- Dysfibrinogenemias usually prolong the TT and are suspected if the functional test (clottable fibrinogen) is disproportionately low compared with an immunologic measurement of fibrinogen.
- 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 antithrombin in a manner similar to heparin. The Reptilase time will be normal in these patients.

Fibrinogen assays

The Clauss method is a modified TT in which fibrinogen rather than the thrombin is limiting. The time to clot formation is 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.

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 antithrombin or direct thrombin inhibitors.

Global hemostasis test

Thromboelastography involves monitoring the viscoelasticity properties of whole blood during clot initiation, contraction, and lysis. Two commercial instruments: TEG (Haemonetics, Braintree, MA) and Rotem (Durham, NC) are available in the United States. 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, fibrinogen deficiency, thrombocytopenia, and hyperfibrinolysis. Most experience with thromboelastography has been in the liver transplantation and cardiopulmonary bypass surgery settings, where rapid point-of-care hemostasis information is used to select blood component replacement products. Modest clinical research has been done with this technology in other medical settings to evaluate patients' bleeding or thrombotic risk, but thromboelastography is not ready for general use as a diagnostic test.

von Willebrand factor assays

Endothelial cells and megakaryocytes synthesize von Willebrand factor (vWF) molecules, which undergo dimerization and subsequent linkage of dimers to form vWF multimers before secretion into 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 two activities: adhesion to connective tissue and platelets and binding factor VIII. Although most deficiencies of vWF (vWD) are congenital, vWF deficiency also can be acquired—a condition known as the acquired von Willebrand syndrome (AvWS). AvWS often is associated with lymphoproliferative disorders, particularly monoclonal gammopathy of unknown significance (MGUS), autoimmunity, hypothyroidism, and severe aortic stenosis, as well as with left-ventricular assist devices (LVAD). 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 frequently is indicated to confirm abnormal results before diagnosing a patient with vWD. See Chapter 8 for additional information regarding clinical presentation, classification, and management of vWD.

Initial testing for vWD

Global tests of primary hemostasis, including bleeding time and PFA-100® (Siemens) closure times, lack both sensitivity and specificity for vWD, and aPTT is an indirect and potentially insensitive screening test for low-factor VIII activity. vWF antigen concentration (vWF:Ag), vWF-mediated agglutination of platelets (vWF:RCo) or 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, activity, and factor VIII levels as low as 35%-40%. It is reasonable to consider vWF levels in the range of 30% to 50% as risk factors for mild bleeding tendency rather than an 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 functional activity is another matter. The most widely used method is the ristocetin cofactor assay (vWF:RCo), performed on a platelet aggregometry instrument, which assesses vWF binding to platelet GPIb/IX/V complex. Ristocetin, an antibiotic, binds to vWF, causing a change in conformation that mimics the effect of high shear stress in vivo to expose the platelet-binding domain. Control platelets bind to the modified vWF multimers, causing agglutination and increased light transmission. The vWF:RCo activity is sensitive both to quantitative deficiencies of vWF (type 1 deficiency) and to mutations causing reductions in large and medium 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 11-11 and 11-12). The vWF:RCo assay is labor intensive, and imprecise, leading to the development of alternative methods to assess adhesive activity, including binding to immobilized collagen, immobilized platelet GPIb to capture vWF, and automated immunoturbidity assays using lyophilized platelets and ristocetin. An automated immunoturbidity assay using latex particles coated with monoclonal antibodies to the vWF GPIb-binding domain compares favorably with vWF:RCo activity for detection of vWD.

Specialized testing to classify vWD

Dismissing a diagnosis of vWD or confirming a diagnosis of type 1 or type 3 vWD usually can be accomplished by

Table 11-12 Assays for vWD classification.

vWD type	vWF Activity	vWF Antigen	RIPA	FVIII	Multimers
Type 1	↓	↓	↓	↓	Nl pattern
Type 2A	↓↓	↓	↓↓	↓	↓ Large and intermediate
Type 2B	↓↓	↓	↑↑↑	↓	↓ Large
Type 2M	↓↓	↓	↓↓	↓	Normal
Type 2N*	NI	NI	NI	↓	Normal
Type 3	↓↓↓	↓↓↓	↓↓↓	↓↓↓	Undetectable

* FVIII low;

RIPA = ristocetin-induced platelet aggregation;

vWD = von Willebrand disease; 2N = Normandy variant of vWD.

reviewing vWF:Ag, vWF activity, and factor VIII activity results. vWF activity or factor VIII activity much lower than vWF:Ag, however, is an indication for more specific testing. vWD multimer analysis provides qualitative information by identifying structural abnormalities that correlate with qualitative defects in vWF adhesion (Figure 11-7). Electrophoresis of

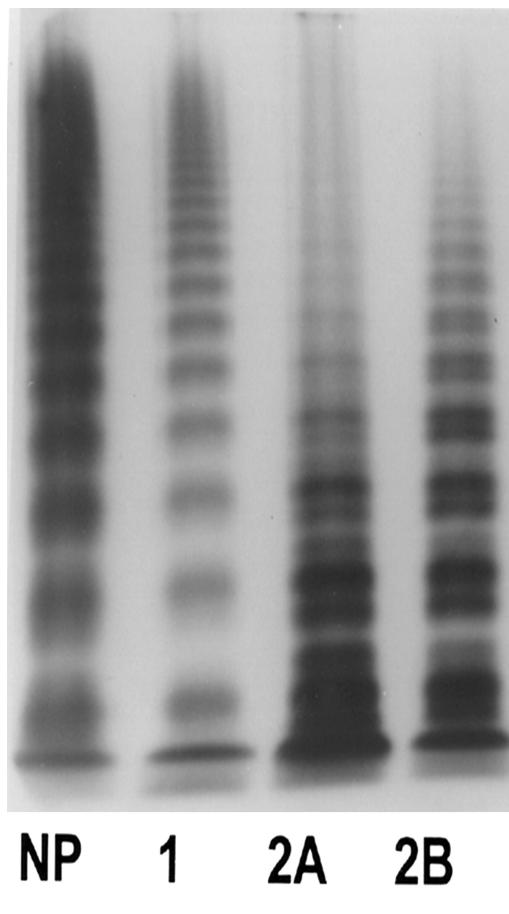


Figure 11-7 von Willebrand multimer patterns. NP = normal plasma; 1 = type 1 von Willebrand disease (vWD) with normal bands but decreased staining intensity; 2A = type 2A vWD with loss of large and intermediate multimers; 2B = type 2B vWD with loss of large multimers.

plasma through low-concentration agarose gel separates vWF multimer bands by size, which are detected with radiolabeled, 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 large and intermediate-size bands (consistent with type 2A, type 2B, and platelet-type vWD).

The ristocetin-induced platelet aggregation assay (RIPA) is a variation on the vWF:RCO activity 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, while change in light transmission is monitored as platelets bind to vWF and aggregate (Figure 11-8). 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 8-10 to 1. Although the disorders have similar clinical presentations and inheritance is autosomal dominant, they require different types of hemostasis replacement products (vWF concentrate vs. platelets, 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. 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 deficiences lacking X-linked inheritance pattern consistent with

hemophilia A may be homozygous for type 2N vWD (decreased vWF binding affinity for factor VIII) or compound heterozygous (type 1/2N). Decreased binding of control factor VIII to the patient's immobilized vWF in an enzyme-linked immunosorbent assay (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 and fibrinolytic pathway defects is rare, yet it 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 will disrupt the clot if fibrin has not been cross-linked by factor XIIIa. Alternative quantitative assays are available to directly quantify factor XIII concentration and activity. Global screening tests of the fibrinolytic system include the euglobulin clot lysis time (ECLT), which measures the time to lyse a fibrin clot in the absence of plasmin inhibitors, and the whole blood clot lysis time (see thromboelastography). Congenital hyperfibrinolysis is due to deficiencies of tissue plasminogen activator (tPA) or plasmin natural inhibitors, and laboratory evaluation requires a panel of analytes, including plasminogen, plasminogen activator inhibitor 1 (PAI-1) activity, and antigen, tPA antigen, and α_2 -antiplasmin activity.

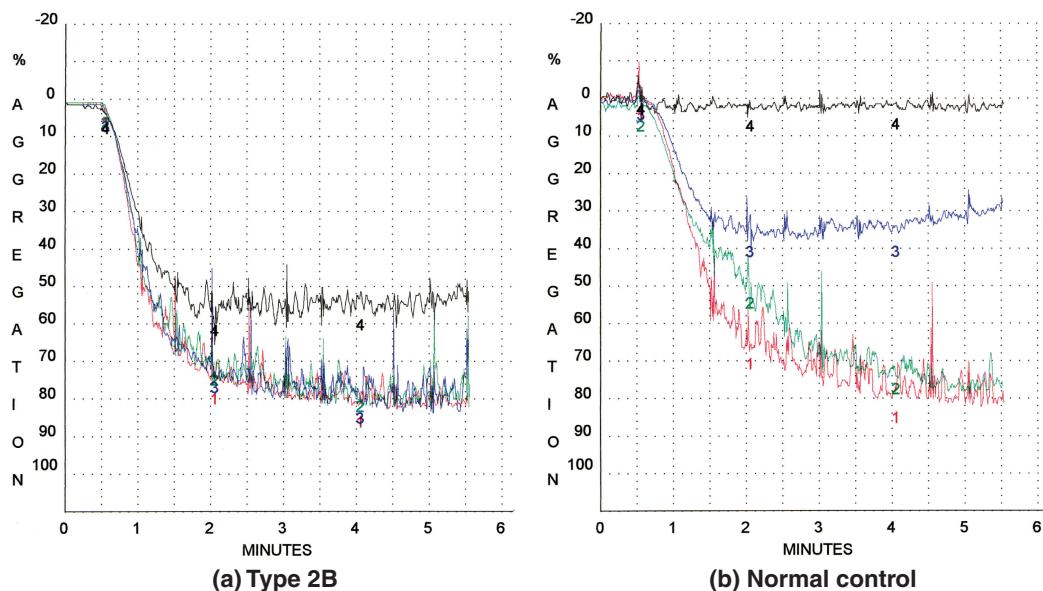


Figure 11-8 Examples of platelet-rich plasma aggregation responses to a range of ristocetin concentrations [1 = 1.5, 2 = 1.2, 3 = 0.9, 4 = 0.6 (mg/mL)]. (a) Type 2B vWD patient showing >50% aggregation with all ristocetin concentrations; (b) Normal control demonstrating concentration-dependent aggregation.

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 the disseminated intravascular coagulation (DIC) process associated with acute promyelocytic leukemia and rarely with solid tumors, including prostate cancer. Laboratory support 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 (see Chapter 2 for more details). 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.

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 testing usually are not informative in these cases. Platelet function testing is technically demanding, time consuming, and poorly standardized, although efforts are under way to develop guidelines for performing and interpreting these studies. The hematologist should be aware that labs use two different platforms to analyze platelet aggregation: instruments that are used to test platelet-rich plasma and instruments that use whole blood samples (whole blood aggregometry [WBA]). Testing is performed on aliquots of

citrated 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 11-9 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 WBA 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 disease, 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, but these tests may be accessible through research laboratories.

Global primary hemostasis screening tests

The template bleeding time is an invasive test, fraught with difficult-to-control technical and patient variables, that lacks specificity and sensitivity 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 5-10 minutes.

Most laboratories have discontinued performing template bleeding times and substituted automated in vitro screening methods, which do not require an incision and provide precise results from samples of blood collected in citrate, yet have similar limitations. The PFA-100 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 hole in a membrane coated with collagen and ADP (COLL/ADP) or collagen and epinephrine (COLL/EPI). vWF multimers bind

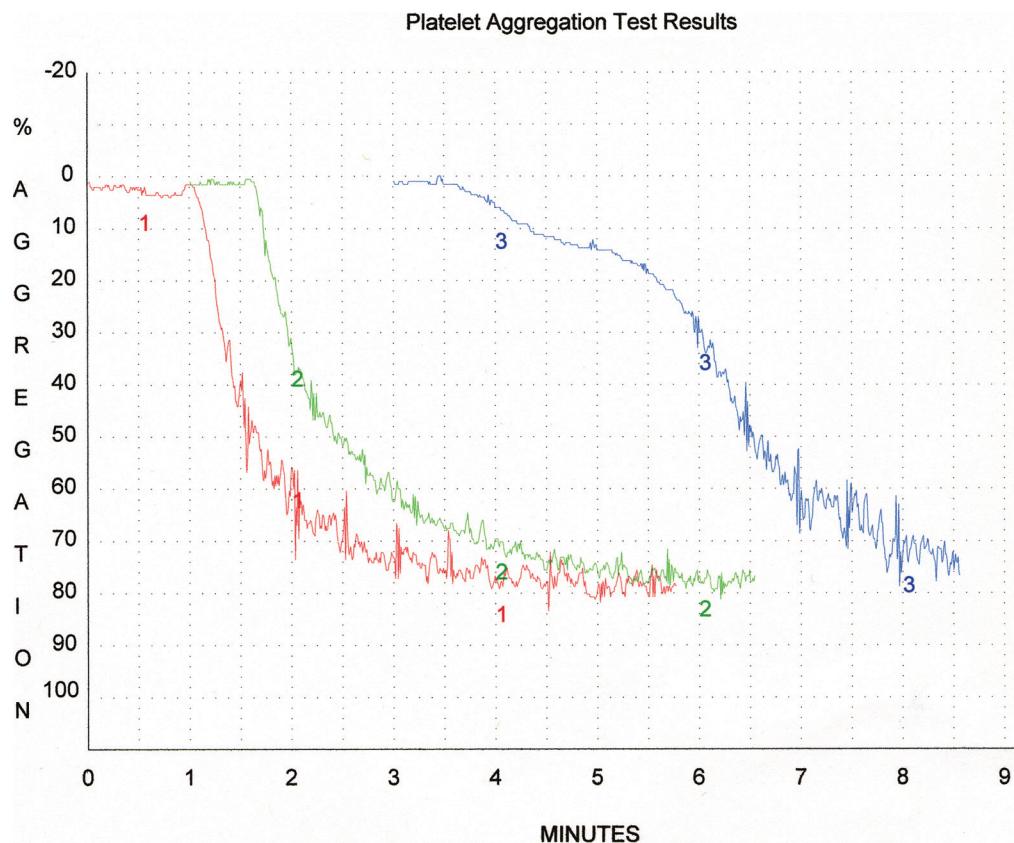


Figure 11-9 Representative platelet aggregation curves performed on normal platelet-rich plasma.
1 = collagen 5 µg/mL,
2 = ADP 5 µg/mL,
3 = epinephrine 5 µM/mL.

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 55–137 seconds and 78–199 seconds for COLL/ADP and COLL/EPI cartridges, respectively. Prolonged PFA-100 closure time is 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 × 10⁶/µL. 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 HIT

HIT is a clinical diagnosis supported by serologic and functional assays. In vitro functional assays monitor activation of normal 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 to monitor carbon¹⁴-labeled serotonin secretion from control platelets. In Europe, heparin-induced platelet aggregation 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 ELISA assays detect antibodies recognizing immobilized PF4 bound to heparin or polyvinylsulfonate complex. Although sensitive, HIT ELISA results are nonspecific, detecting antibodies incapable of activating platelets *in vitro* or causing thrombocytopenia and thrombosis *in vivo*. The positive predictive value of a positive PF4 ELISA result alone to confirm a diagnosis of HIT is a low, and if used as the only criterion, a positive PF4 ELISA results in the overdiagnosis of HIT. Growing evidence supports three 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 called the 4Ts (*thrombocytopenia, timing, thrombosis, and 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 a FP test, such as patients who have had a cardiopulmonary bypass procedure. Second, identifying only IgG instead of a combination of IgG/IgM/IgA PF4 antibodies improves the specificity of a positive PF4 ELISA with a slight impact on sensitivity. Finally, ample evidence suggests that the higher a PF4 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 PF4 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.

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, <5%–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 methods that currently are 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 or fluorescence resonance energy transfer (FRET) 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 and 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 until ADAMTS13 activity and inhibitor results return because they improve diagnostic specificity at the expense of sensitivity. Persistently low ADAMTS13 activity and positive inhibitor titer are predictors of relapse during remission.

Assays for thrombophilia

Inherited deficiency of one or more of the identified natural inhibitors of coagulation (antithrombin, 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 anticoagulation therapy, testing for nonmolecular 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 antithrombin, PC, and PS, genotyping is not routinely performed.

Antithrombin deficiency

The most sensitive screening tests for antithrombin deficiency are chromogenic activity assays designed to quantify antithrombin inhibition of factor Xa or IIa in the presence of unfractionated heparin. Abnormal low-antithrombin activity results require measurement of antithrombin antigen to classify the deficiency as type I (activity = antigen) or type II (activity < antigen). Type I antithrombin 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 antithrombin heparin-binding defects. Although type IIb is associated with a low risk of thrombosis, progressive antithrombin activity assays are not readily available and typically are not performed.

PC deficiency

The preferred screening tests for PC deficiency are chromogenic assays. PC is activated with a snake venom. 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, factor V Leiden, inhibitory antibodies, and anticoagulants. An abnormal low-PC activity result requires measurement of the PC antigen to classify the deficiency as type I (activity = antigen) or type II (activity < antigen).

PS 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 mutation

Two autosomal inherited coagulation factor variants increase the risk for VTEs; these are factor V G1691A (factor V Leiden) and prothrombin G20210A. Several sensitive commercial clot-based screening assays for factor V Leiden mutation demonstrate a resistance of factor Va cleavage by aPC in the presence of factor V Leiden mutation. Coagulation testing, activated with aPTT, PT, or Russell 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 determine whether they are heterozygous or homozygous for factor V Leiden. 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

The APS is an important acquired thrombotic condition. Consensus-based criteria have been developed for the investigational diagnosis of APS. These criteria require a combination of clinical conditions (unexplained venous or arterial thromboembolic events, pregnancy complications) and persistent laboratory evidence of autoantibodies that recognize epitopes on selected proteins associated with phospholipids and identified by coagulation-based (LACs) or serologic-based (cardiolipin and β_2 GPI antibodies) testing. LACs are heterogeneous antibodies that interfere with in vitro clotting assays. Indirect evidence for the presence of a 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 recommend performing two sensitive LAC tests in parallel—one aPTT-based test and one Russell 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 ($>10,000/\mu\text{L}$) 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 will have elevated INRs before starting warfarin. Chromogenic factor X activity is an alternative to the INR for therapeutic anticoagulation monitoring (target 20%-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 one 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 autoantibody 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 ELISA testing for anticardiolipin (aCL) and anti- β 2GPI (a β 2GPI) 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 for aCL and a β 2GPI 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 a β 2GPI results to be clinically important.

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Transfusion medicine

Karen Quillen and Suzanne Bakdash

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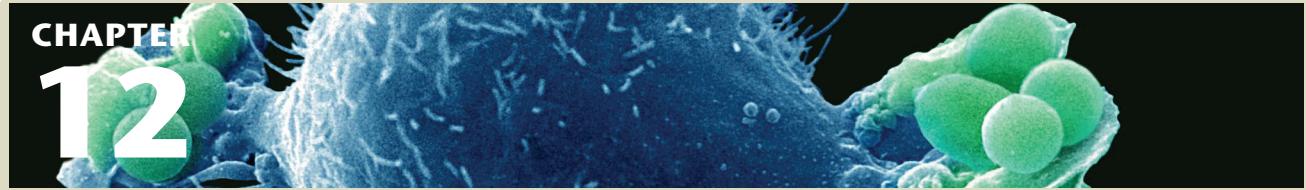
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CHAPTER
12



Transfusion medicine

Karen Quillen and Suzanne Bakdash

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Introduction

Transfusion medicine encompasses the field of transfusing blood components for the appropriate indications after pre-transfusion compatibility testing, recognizing the current risks of transfusion and the presenting manifestations of transfusion reactions. Clinical settings unique to specific hematology populations, such as sickle cell disease and hematopoietic stem cell transplant (HSCT) recipients, are of particular relevance to the hematologist practitioner. Apheresis includes therapeutic apheresis, which removes a constituent of whole blood contributing to disease pathogenesis, and peripheral blood stem cell (PBSC) harvesting, which involves mobilizing hematopoietic progenitor cells from the bone marrow into the blood and subsequent collection by apheresis for allogeneic or autologous PBSC transplantation. Much of transfusion practice was empiric for many years, but recent landmark clinical trials have provided a more evidence-based approach.

Red blood cell transfusion

ABO system

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 on the sub-terminal galactose 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 Caucasian 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%) in this population.

The ABO gene locus is on chromosome 9. The A and B genes encode transferase enzymes that covalently attach the specific terminal saccharide moiety to the subterminal galactose. The large number of A and B transferase polymorphisms has precluded the development of reliable genotyping methods for the determination of ABO blood group. In most cases, the gene underlying the group O phenotype is identical to the A transferase gene except for a single base-pair deletion that leads to premature termination of translation. Blood group O most likely evolved in Africa because it provides a selective advantage against severe malaria. Individuals with blood group O have lower levels of von Willebrand factor (vWF), which needs to be taken into consideration in the diagnosis of mild type I von Willebrand disease. Conversely, nongroup O individuals have a greater risk of venous thromboembolism attributable to the higher levels of vWF and FVIII.

Healthy people past infancy nearly always produce immunoglobulin M (IgM) anti-A or anti-B antibodies, also known as isoagglutinins, directed against the respective ABO antigens that are not present on their own cells. Thus, group O individuals have so-called naturally occurring circulating anti-A and anti-B antibodies, group A individuals have anti-B antibodies, group B individuals have anti-A antibodies, and

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Off-label drug use: Dr. Quillen: Recombinant activated Factor VII for refractory bleeding. Dr. Bakdash: Recombinant activated Factor VII for refractory bleeding.

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, and death. In blood group O individuals, anti-A and anti-B antibodies may be of both the IgM and IgG isotypes. An additional antibody, anti-A,B, which cross-reacts with both type A and type B RBCs also is found in type O individuals and is predominantly of the IgG isotypes. 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.

Variations in the strength of ABO antigen expression are occasionally clinically significant. For example, so-called type A₂ individuals manifest substantially weaker A antigen expression and occasionally can develop antibodies directed against the common type A RBCs (referred to as A₁) despite the presence of detectable A carbohydrate. The differences in A antigen expression among RBCs of differing A subgroups are qualitative as well. Consequently, the anti-A₁ antibodies made by non-A₁-expressing individuals are not self-reactive. Anti-A₁ antibodies typically bind only to A₁-positive RBCs at nonphysiologic temperatures; however, when reactive at 37°C or the anti-human globulin (AHG or Coombs phase), only A₂ or O RBCs should be used for transfusion. Subgroups of B antigen exist as well but are encountered much less frequently.

In addition to being expressed on RBCs, ABO antigens also are expressed on endothelial cells. ABO compatibility typically is required for solid organ transplantation to avoid the risk of ABO antibody-mediated acute humoral rejection. For example, if a blood group O recipient is transplanted with a solid organ from a blood group A donor, the recipient's anti-A antibody can mediate humoral rejection and destruction of the transplanted organ. There are few exceptions to the requirement for ABO compatibility in solid organ transplantation, most of which involve donors of the A₂ subgroup. ABO compatibility is not required for hematopoietic stem cell (HPSC) transplantation. 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 their isoantibody production in the recipient, potentially resulting in donor ABO antibody-mediated hemolysis of the recipient's RBCs. For example, transplantation of a liver from a group O donor would be acceptable for a group A recipient because the

recipient's anti-B antibodies will not cause humoral rejection of the transplanted organ. 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 RBCs.

Rh system

Clinically, the Rh blood group system is second in importance to the ABO system. Rh antigens are proteins and, unlike the ABO system, antibodies to Rh antigens rarely are present unless a person has been immunized by pregnancy, transfusion, or stem cell transplantation. The Rh system is a collection of 50 different antigens encoded by two genes, designated *RHD* and *RHCE*. *RHD* and *RHCE* are 97% identical, include 10 exons, and evolved from a gene-duplication event on chromosome 1p34-36. Individuals who are referred to as Rh-positive express the D antigen; approximately 85% of whites and 92% of blacks are RhD positive. Individuals who are Rh negative do not express D, either because they have a complete deletion of the *RHD* gene (European descent) or have nonfunctioning *RHD* resulting from premature stop codons, gene insertions, or other causes (Asian and African descent). On a protein level, D and CE are 417-amino acid, nonglycosylated transmembrane proteins that are predicted to span the membrane bilayer 12 times. Whereas one protein carries the D antigen, and identifies an individual as Rh positive or Rh negative, the other protein carries various combinations of the CE antigens (eg, ce, cE, Ce, or CE). D differs from CE by 32-35 amino acids, depending on which of the four possible forms of CE protein is present. 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, in which none of the previously listed Rh antigens are expressed on the cell surface, is associated with stomatocytic erythrocytes and low-grade hemolytic anemia. Many physiologic defects have been described in Rh_{null} cells, but the function of the Rh proteins themselves only now is becoming appreciated. Initial clues to the putative function of Rh proteins came from amino acid sequence analysis that linked them to the family of ammonium (NH₄⁺) transporters present in bacteria, fungi, and plants. Rh-associated glycoprotein (RhAG) provides a channel for neutral gases NH₃ and CO₂. The Rh polypeptides D and CE facilitate the assembly of major transport proteins in the RBC membrane, such as band 3.

Blood routinely is typed and matched for the presence of the D antigen for two primary reasons. First, as noted, the D antigen is highly immunogenic, and approximately 80% of

D-negative individuals exposed to D become alloimmunized to D. Second, before the advent of prophylaxis with Rh(D)-immune globulin (eg, RhoGAM; Ortho-Clinical Diagnostics, Raritan, NJ), anti-D commonly caused HDFN. Prevention of immunization to the D antigen in women of childbearing potential continues to be extremely important, and failure of prophylaxis continues to account for a significant percentage of cases of HDFN. When a dose of Rh(D)-immune globulin (typically 300 mcg) is administered at 28 weeks of pregnancy and again at delivery (if the newborn turns out to be Rh positive), Rh(D)-immune globulin is 99.9% effective in preventing maternal alloimmunization to D. The exact mechanism by which Rh(D)-immune globulin prevents sensitization in the Rh-negative individual when exposed to Rh-positive RBCs remains unknown. It has been proposed that Rh-positive fetal RBCs coated with Rh(D)-immune globulin in the maternal circulation serve to cross-link surface immunoglobulin to inhibitory Fc receptors on maternal naïve B-cells to render them anergic, although this mechanism has yet to be proven experimentally. The other major antigens of the Rh system—C, c, E, and e—also are relatively potent immunogens and can cause severe HDFN, albeit at lower frequencies than D. No immune globulin preparations are available for the prevention of alloimmunization to Rh antigens other than D.

As is the case with the ABO system, variations in the strength of expression of Rh antigens can be clinically significant. In the case of D, clinical significance depends on the etiology of its reduced expression. Two types of scenarios are worthy of discussion. The first is strictly quantitative in nature—that is, RBCs express a structurally normal D protein, but they do so in reduced amounts. Individuals with this weak-D phenotype can be considered Rh positive for all intents and purposes; that is, they are immunologically tolerant to D and thus can receive wild-type Rh-positive RBCs. Likewise, if pregnant with an Rh-positive fetus, they would not need to receive Rh(D)-immune globulin to prevent D sensitization. For blood donors, reagents and techniques to detect weakened D expression are paramount so that such units are labeled Rh positive to avoid sensitizing patients who truly are Rh negative. Many transfusion service laboratories choose Rh-typing reagents and methods that will not detect weak D phenotypes. This creates a situation in which an individual may be classified as Rh negative as a patient but Rh positive as a blood donor.

The second scenario is one in which an individual's D antigen is qualitatively different than wild-type D, which, in turn, can lead to a quantitative reduction in the level of cell surface expression. In many of these cases, the qualitative alteration is the result of the replacement by homologous recombination of one or more exons of the *RHD* gene by the corresponding exon(s) of the nearby *RHCE* gene. As a consequence, the RBCs of such individuals, referred to as *partial D*

cells, express D proteins that are chimeric in nature and are made up of pieces of CE. The immunologic ramification of this genetic alteration is that such individuals may type as Rh positive (depending on the particular formulation of anti-D typing reagent used); however, after being transfused with true Rh-positive RBCs, may make anti-D alloantibodies to the D epitopes they lack. Such antibodies may be clinically significant in terms of their ability to cause hemolysis or HDFN. Therefore, such individuals should be transfused with Rh-negative RBCs and, if pregnant, should be given Rh(D)-immune globulin. In practice, these individuals typically are identified only after they have formed anti-D despite typing as Rh positive, or they present with inconsistent Rh typing results with different reagents that recognize different epitopes of the D polypeptide.

Other protein antigen 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, and MNSs systems (Table 12-1). 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 can form several antibodies simultaneously, which can limit the availability of donor blood. 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 in patient serum during pretransfusion testing. In the acute phase of alloimmunization to nonself protein antigens, T-cell-independent IgM antibodies may appear first, which subsequently isotype switch to IgG.

Antibodies to certain blood group antigens are seen in patients more commonly than other antibodies. This is not simply a function of the inherent antigenicity of the antigen but rather is a consequence of the relative frequency of the antigen in the population. For example, the K1 antigen of the Kell blood group system (often referred to as just K) is expressed on the RBCs of approximately 10% of blood donors and patients. Therefore, there is a sizable proportion of individuals who are capable of mounting an immune response to K1 (90%) and a reasonable chance of one of these individuals receiving a unit of K1-positive cells during a transfusion. Consequently, anti-K1 antibodies are commonly identified antibodies.

Anti-K1 antibodies can cause accelerated clearance of transfused cells as well as significant HDFN. Unlike HDFN due to anti-Rh blood group antibodies, the anemia associated with HDFN due to anti-K1 appears to have a

Table 12-1 Commonly occurring red blood cell (RBC) antigens of clinical significance.

RBC antigen system	Molecule expressing antigen	Function of molecule	Antibody immune/naturally occurring	Hemolytic transfusion reaction from antibody	Hemolytic disease of the newborn from antibody
ABO	Glycoprotein or glycolipid	Unknown	Naturally occurring	Yes, acute	Yes, mild (IgG form of 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 neprilysin (M13) family of zinc metalloproteases	Immune	Yes, delayed	Yes, often severe; hypoproliferative component
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 (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)	Variable

hypoproliferative component as well, because of the expression of Kell antigens on fetal hematopoietic progenitor cells. From a clinical management standpoint, the effects of maternal anti-K1 antibodies on the fetal RBC precursors that express K1 have rendered the use of the maternal anti-K1 titer less reliable as an indicator of fetal status than titers in Rh antibody-associated disease. In these cases, more invasive monitoring often is used (eg, amniotic fluid analysis, percutaneous umbilical blood sampling). The Kell blood group system is interesting for several other reasons as well. The protein bearing the Kell system antigens is related structurally to a number of metalloproteases, including CALLA (common acute lymphoblastic leukemia antigen, or enkephalinase) and endothelin-converting enzyme. In addition, weakened expression of inherited Kell antigens may be associated with a rare phenotype, called the McLeod phenotype, because of a deficiency of a protein called Kx. Unlike Kell, Kx is encoded by the XK gene on the X chromosome, near the locus affected in the X-linked form of chronic granulomatous disease (CGD); therefore, in some families, the McLeod phenotype is associated with CGD. McLeod phenotype cells are classified as *spur cells* (acanthocytes) because they contain sharp, irregular cytoplasmic projections. Absence of the Kx protein is responsible for most instances of congenital acanthocytosis associated with neurologic dysfunction.

Another important protein antigen system is the Kidd system, which 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). The pathophysiology of DHTRs will be discussed later, but briefly note the following: (i) An individual is sensitized via transfusion, pregnancy, or HSCT; (ii) The antibody titer decays over time, such that the antibody becomes undetectable by standard serologic techniques at the time that the antibody screen is performed; (iii) Because of the apparently negative antibody screen, the patient is transfused with an ABO and RhD-compatible unit; (iv) Upon reexposure to the Kidd antigen, the recipient develops a rapid anamnestic antibody response, which results in clinically significant hemolysis several days after the transfusion. Although antibodies to other blood group antigens can cause DHTRs, the severity of DHTRs resulting from antibodies of the Kidd blood group system is compounded by the fact that Kidd antibodies, although IgG, are excellent at fixing complement resulting in the more clinically significant intravascular form of hemolysis.

Alloantibodies that develop to antigens in the Duffy blood group system may cause hemolytic transfusion reactions, which are acute or delayed, and cause HDFN. Their clinical significance in both scenarios can vary from mild to severe. The Duffy glycoprotein itself is structurally related to

chemokine receptors that bind interleukin (IL)-8, monocyte chemotactic protein-1 (MCP-1), and other chemokines, although its function on RBCs is not clear. It may impart RBCs with the ability to scavenge excess chemokines from the circulation. The Duffy glycoprotein also serves as a receptor for the malarial parasite *Plasmodium vivax*, which explains the significant variability in the expression of Duffy antigens between whites and blacks. There is some evidence that the Duffy glycoprotein is expressed on nonerythroid tissue and represents a minor histocompatibility antigen in kidney transplantation.

The MNSs blood group system is highly complex, including 46 antigens that reside on one or both of the major RBC membrane glycoproteins—glycophorin A (GPA) and glycophorin B (GPB). The red cell antigens M and N reside on GPA, which is critical to the invasion of RBCs by *Plasmodium falciparum*. Alloantibodies to the M and N antigens are generally 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 GPB, are clinically significant IgG antibodies that can cause hemolytic transfusion reactions and HDFN.

Other carbohydrate antigen systems

Carbohydrate antigen systems other than the ABO system are rarely significant in clinical transfusion practice, but some issues of pathophysiologic importance are of interest.

The Lewis antigens (Le^a and Le^b) and the P_1 antigen are common targets of cold-reacting IgM alloantibodies. 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. Lewis antigens expressed on gastric mucosa serve as receptors for *Helicobacter pylori*. People 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. Interestingly, recent studies have suggested that the presence or absence of certain members of the $P/P_1/P^k$ blood group system on mononuclear cells may modulate susceptibility to HIV infection.

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; cold agglutinin disease). The expression of the Ii antigen system is age dependent. In newborns, the predominant allele is the i antigen, which

includes linear repeats of N-acetylgalucosamine and galactose (N-acetylgalactosamine). In older individuals, 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 erythrocytes express i weakly and I strongly.

As is the case with antibodies directed against ABO antigens, antibodies directed against other carbohydrate antigens tend to be of the IgM isotype. One exception to this rule is found in the syndrome of paroxysmal cold hemoglobinuria (PCH), in which Donath-Landsteiner antibodies, which are cold-reacting IgG autoantibodies directed against the P antigen can fix complement on circulating RBCs resulting in intravascular hemolysis. PCH is sometimes associated with syphilis or nonspecific childhood viral infections. Of historical note, PCH is considered the first example of an autoimmune disease and was referred to by Ehrlich as *horror autoxicus*.

Blood group genotyping

Over the past two decades the molecular bases of almost all the major blood group antigens have been elucidated. It is now known that the majority of blood group polymorphisms are caused by simple single-nucleotide polymorphisms in the genes encoding the protein antigens or genes encoding the glycosyltransferases for the carbohydrate antigen systems. 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 then evolved to permit the same analysis on the basis of free fetal DNA in maternal plasma, eliminating the risk of amniocentesis altogether. 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 is the standard of care. In Caucasians, the RhD-negative phenotype results from a deletion of the *RHD* gene on chromosome 1. In African populations, a significant portion of RhD-negative individuals do not have a complete gene deletion. This difference is important for genotype-phenotype correlation of DNA-based RhD typing assays. In addition, D-variant alleles are

more common in people of African descent. Molecular classification of D variants allows for the recognition of partial D phenotypes—that is, individuals who type as RhD positive but are at risk of developing anti-D if transfused with wild-type Rh-positive blood or pregnant with a wild-type Rh-positive fetus. In one study in Brazil, the D-variant antigens *DIIIa* and *DAR* were found in 6% of sickle cell patients, of whom some already had made anti-D. Sick cell patients also have a higher prevalence of *RHCE* variants, which can seriously complicate transfusion management.

In the past several years, array-based high-throughput approaches to blood group genotyping have become commercially available. One system is an oligonucleotide array with multiple probes corresponding to allelic pairs of blood group-specific SNPs. Genomic DNA is extracted from leukocytes in whole blood, DNA sequences flanking each SNP are amplified by multiplex PCR, and PCR products then are labeled and hybridized to probes on the glass array. Another system uses color-coded beads attached to oligonucleotide probes. The latter system can be configured for platelet antigen genotyping and class I HLA genotyping. These molecular platforms have been adopted in many blood centers, particularly in Europe.

Blood group genotyping is invaluable in multitransfused patients whose erythrocytes represent a mixture of donor and recipient and as such cannot be accurately phenotyped using conventional serologic techniques. As an example, genotyping to determine that a patient is homozygous for the *Jkb* allele (therefore lacking the *Jka* antigen expression) would focus the investigation of a suspected delayed hemolytic transfusion reaction to the possibility of an anti-*Jka*. Conventional serologic typing in this instance most likely would identify the patient's erythrocytes to contain a mixture of *Jka*-positive cells (the transfused donor cells) and *Jkb*-positive cells (the patient's own). Blood group genotyping is particularly useful in multitransfused patients at high risk of alloimmunization, such as patients with sickle cell disease. Some blood centers have screened blood donors by genotyping to offer blood units that are “extended antigen matched” (beyond ABO/Rh) to patients with sickle cell disease to prevent alloimmunization. Patients with warm AIHA whose erythrocytes are coated with IgG (leading to a positive direct Coomb's test) are difficult to antigen type with conventional antisera for many protein antigens and also may benefit from blood group genotyping.

Collection and storage of RBCs

Most RBCs collected in the United States are obtained from healthy volunteer donors, although collection of autologous RBCs and RBCs from *directed donors* also occur. Most units of whole blood collected from volunteer donors are fractionated

into one or more transfusible components, including packed RBCs, platelets, fresh frozen plasma (FFP), and others.

RBCs can be stored in plasma or in a variety of additive solutions (ASs). One commonly used AS for RBC storage is AS-1. AS-1 contains glucose, adenine, and mannitol. Largely due to advances in storage techniques, RBCs have the longest shelf life of any of the three major transfusible cell types (RBCs, platelets, and granulocytes). RBCs are stored routinely for up to 42 days at 4°C in currently available storage media. Techniques for freezing RBCs impart a shelf life of 10 years or greater in special situations, such as rare blood types.

Cold storage of RBCs has long been known to induce biochemical changes in the RBC component, such as decreased 2,3-diphosphoglycerate (2,3-DPG levels), which are mostly reversible *in vivo* after transfusion. A landmark single-center retrospective study in cardiac surgery patients suggested that transfusion of RBCs stored for >2 weeks was associated with increased postoperative complications and mortality. This has sparked ongoing randomized controlled trials (RCT) to prospectively investigate differences in outcomes after the transfusion of fresher versus older stored RBCs in cardiac surgery (the RECESS trial) and in intensive care unit (ICU) patients with respiratory failure (the ABLE trial). Data from an RCT in preterm neonates (the ARIPI trial) showed no difference in outcomes when fresh versus standard-age blood was used. Possible hypotheses of adverse outcomes include cell-free hemoglobin in older stored RBC units acting as a scavenger of nitric oxide and leading to vasoconstriction, and the oxidant, proinflammatory, and immunomodulatory effects of iron derived from damaged transfused RBCs.

Clinical transfusion of RBCs

Clinical case

A 29-year-old man with chronic renal failure has a hemoglobin (Hb) of 6.7 g/dL and is seen in the emergency room for flu-like symptoms and cough with normal heart rate and blood pressure. The attending physician orders a blood transfusion, but the patient states that he usually has this degree of anemia, and he recently has begun therapy with recombinant darbepoietin through his nephrologist. He has been able to conduct his normal office duties without difficulty and routinely drives himself to the outpatient dialysis unit.

From the perspective of the clinician, the starting point for transfusion is deciding whether it is indicated. The next most important consideration for ensuring the safe administration of RBCs is definitive identification of the patient. Specifically, it is imperative that the labeling of the type and cross-match specimen as well as the definitive identification of the unit to be transfused occur at the bedside, and not outside the patient's room.

The primary goal of erythrocyte transfusion is to improve the oxygen-carrying capacity of the blood in patients with anemia. Although not solely indicated for this purpose, erythrocyte transfusion can provide one component of the overall management of hypovolemia in patients with intravascular volume depletion because of acute blood loss, systemic inflammatory syndrome with third spacing, or other etiologies. Table 12-2 summarizes the major available RBC products and their respective indications.

Numerous compensatory mechanisms exist to maintain oxygen delivery in the face of anemia. These mechanisms include increased pulse rate and cardiac contractility, peripheral vasodilatation, increased oxygen delivery to tissues resulting from decreased oxygen affinity of hemoglobin because of increased erythrocyte 2,3-DPG concentration and decreased plasma pH, and altered oxygen consumption and utilization within the tissues. Studies relating hemoglobin level to anaerobic threshold indicate that in healthy people, 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. When cardiopulmonary disease or other disorders affecting oxygen availability or consumption exist, the hemoglobin level at which anaerobic metabolism begins to occur may be increased.

As a result of these factors, there is no fixed hemoglobin target for the transfusion of RBCs. A relatively young,

ambulatory, and otherwise-healthy individual involved in a sedentary occupation may tolerate a blood hemoglobin concentration of 6 g/dL or less without particular difficulty, as long as the anemia was gradual in onset. In contrast, an older individual with a severe cardiac or pulmonary disorder or an individual with acute-onset anemia due to traumatic blood loss may require a higher blood hemoglobin concentration to maintain clinical stability. In general, hemodynamically stable patients in the ICU should not be transfused unless their hemoglobin is less than 7 g/dL. A recent study found that such a restrictive transfusion strategy improved outcomes in selected patients with acute upper-gastrointestinal bleeding. The evidence regarding a restrictive or liberal transfusion strategy in patients with acute coronary syndromes is unclear.

Another factor that may influence the patient's target hemoglobin level is concurrent thrombocytopenia. Both clinical and laboratory evidence suggests that the hemorrhagic defect associated with thrombocytopenia may be exacerbated by moderate to severe anemia. Therefore, many clinicians attempt to maintain a hemoglobin level of 8 g/dL or higher in patients with severe concurrent thrombocytopenia, for example, in patients undergoing induction therapy for acute leukemia or HSCT. A randomized trial comparing red cell transfusion thresholds in HSCT patients is under way in Canada.

Whole blood is not widely available in the United States, and therefore, packed RBCs are the most frequently used

Table 12-2 Characteristics and indications for various red blood cell 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
Packed red blood cells	250-300 mL; can be stored up to 42 days	To provide increased oxygen-carrying capacity
Leukocyte-reduced packed red blood cells	Contain <10 ⁷ leukocytes per unit	To reduce the incidence of febrile reactions, CMV transmission, HLA alloimmunization, and platelet transfusion refractoriness
Leukocyte-reduced, γ -irradiated packed red blood cells	Leukoreduced and γ -irradiated	To reduce the incidence of febrile reactions, CMV transmission, HLA alloimmunization, platelet transfusion refractoriness, and transfusion-associated graft-versus-host disease
Deglycerolized frozen red blood cells	200 mL	To support patients with rare blood group phenotypes; to prevent anaphylactic reactions in patients with IgA deficiency
Washed red cells	Saline-suspended red cells 200-250 mL	To support patients with severe or recurrent allergic reactions
Pooled platelets*	300-325 mL, 4-8 donors	Prophylaxis of bleeding: platelet count <10,000/uL; treatment of bleeding: platelet count <50,000-100,000/uL
Single-donor platelets*	150-350 mL, 1 donor	Similar to pooled platelets; limits donor exposure (eg, in aplastic anemia)
HLA-matched single-donor platelets*	150-350 mL, 1 donor	Platelet transfusion refractoriness in association with a documented anti-HLA antibody

* Platelet products should be subjected to leukoreduction or γ -irradiation for the same indications as discussed for red blood cells.

CMV = cytomegalovirus; HLA = human leukocyte antigen; IgA = immunoglobulin A.

form of RBCs. For acute blood loss, RBCs are used either alone or in combination with crystalloid solutions or plasma. Washed RBCs are indicated for patients who have had severe allergic or anaphylactic reactions to blood transfusion; the classic example for this indication is that patients with IgA deficiency may have antibody to IgA, which may cause anaphylaxis with transfusion. Although washed erythrocytes have been recommended to reduce complement levels in patients with paroxysmal nocturnal hemoglobinuria (PNH), it is not known to what degree this practice is necessary. Washing rarely is indicated to reduce the extracellular potassium load in adult patients, even those with renal insufficiency, but it may be indicated in the pediatric setting particularly if large volumes of older RBC units need to be transfused to an infant or neonate. Cryopreserved erythrocytes primarily are used for multiply alloimmunized patients who require units of rare blood type.

Cellular blood products, including RBCs and platelets, are contaminated with small numbers of leukocytes sometimes referred to as *passenger leukocytes*. Evidence suggests that passenger leukocytes play an important role in alloimmunization to human leukocyte antigens (HLAs), transmission of cytomegalovirus (CMV) infection, cytokine-mediated febrile transfusion reactions, transfusion-associated graft-versus-host disease (t-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 strong trend toward the use of universal prestorage or poststorage leukoreduction of both RBCs and platelets, particularly in patients who are likely to require prolonged transfusion support. Leukoreduction does not provide adequate protection against t-GVHD (see discussion later in the chapter), so γ -irradiation of all cellular blood products, in addition to leukoreduction, continues to be used in immunosuppressed recipients.

After the clinician's decision to request RBCs for a particular patient and a properly labeled specimen has been obtained with meticulous identification, the next important step in the clinical transfusion of RBCs is selection of the appropriate unit(s). The steps involved in the selection of an RBC unit include A-, B-, and D-antigen typing of the patient's RBCs; screening of the patient's serum for antibodies to clinically significant RBC antigens (called the antibody screen); and performing a cross-match, in which immunologic compatibility between the patient and the prospective RBC unit is assessed (further details of this process are presented in the section "Pretransfusion Testing"). Finding cross-match-compatible blood for individuals who have

been alloimmunized to foreign RBC antigens after prior pregnancies or transfusions, such as multitransfused sickle cell patients, may take hours to days. 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 erythrocytes may be required.

In the clinical case previously described, the attending physician's initial decision to administer RBCs was incorrect, because it failed to consider the fact that young, otherwise healthy individuals with gradual-onset anemia often tolerate low hemoglobin levels without difficulty. Therefore, the case illustrates the importance of using bedside clinical judgment in making transfusion decisions rather than relying on arbitrary hemoglobin cutoffs.

Key points

- The ABO system is the most important determinant of transfusion compatibility.
- Rh compatibility is also necessary because of its high immunogenicity and potential role in hemolytic disease of the fetus and newborn, and delayed hemolytic transfusion reaction.
- Other frequently relevant blood group systems include Kell, Kidd, Duffy, and MNSs.
- There is no fixed threshold for transfusion of RBCs.

Platelet transfusion

The HLA system

Alloimmunization to HLAs is the major cause of immune-mediated refractoriness to platelet transfusion in patients undergoing chronic platelet transfusion therapy. Of the HLA antigens, only 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. The relative insignificance of HLA class II antigens, that is, the HLA-DR, HLA-DP, and HLA-DQ antigen systems, in clinical platelet transfusion practice stands in contrast to the major importance of these systems, particularly HLA-DR, in the selection of donors for HSCT.

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). 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 (CREGs). When an exact HLA-identical platelet donor is not available, blood centers also can use CREGs to locate platelet donors in whom the risk of cross-reactivity between the recipient's antibodies and the donor's antigens may be minimized. For example, if a particular recipient has an anti-HLA-A3 antibody, a donor whose platelets express HLA-A9 would be less desirable than a donor whose platelets express, for example, HLA-A17, because the recipient's anti-HLA-A3 is likely to cross-react with the HLA-A9 antigen on donor platelets, which is in the same CREG as HLA-A3.

Human platelet antigens

In addition to anti-HLA antibodies, antibodies to platelet-specific antigens also may cause platelet transfusion refractoriness. The human platelet antigens (HPAs) arise as a result of polymorphisms involving various platelet membrane glycoproteins. There appear to be important differences in the various HPA allelic frequencies in different ethnic populations. These differences may partially account for differences in the rates of alloimmunization to HPA antigens reported by different investigators. Alloimmunization to HPAs can cause neonatal alloimmune thrombocytopenia (NAIT) and posttransfusion purpura (PTP) and accounts for approximately 8% of platelet transfusion refractoriness in multiply transfused platelet transfusion recipients. In addition, there are case reports of alloimmune thrombocytopenia after HPA-mismatched allogeneic HSCT.

There are a number of well-characterized HPA antigen systems, but alloimmunization is most commonly due to polymorphisms involving HPA-1a/1b system (previously known as the PLA1/A2 system). The HPA-1a/1b system is attributable to 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 HLA-DQB1*0201 in the recipient.

PTP is a syndrome in which transfused platelets are destroyed by HPA alloantibodies through a process loosely analogous to a DHTR, as discussed previously. Following exposure to the HPA antigen in question, however, through RBC or platelet transfusion, what then follows is the apparent immune destruction of the patient's own antigen-negative platelets. The mechanism by which autologous platelets are destroyed in PTP is unclear, although a process involving cross-reactivity of HPA alloantibodies to patient platelets is a favored explanation. From a transfusion perspective, for the patient with a history of PTP, RBC units

should be washed to remove any contaminating potentially alloreactive platelets that could incite an additional episode of PTP. For platelet transfusions, alloantigen-negative platelets should be selected.

Collection and storage of platelets

Two basic types of platelet products are available for clinical use: pooled products and single-donor products. Pooled products are obtained by pooling individual platelet concentrates derived from whole blood units obtained from 4-8 volunteer whole blood donors. The platelet content of pooled platelet products varies depending on the number of units in the pool and various technical factors. A pooled product derived from 6 units of whole blood (commonly known as a *6-pack*) typically contains at least 3×10^{11} platelets, which is sufficient to raise the peripheral blood platelet count by at least 20,000-30,000/ μL in the average-size adult recipient.

The second basic category of platelet products is single-donor platelets (SDPs). Unlike pooled platelet concentrates, which are derived from multiple volunteer donors' whole blood, SDPs are collected from single donors using continuous centrifugation plateletpheresis techniques in which most of the RBCs and plasma are returned to the donor at the time of collection. Plateletpheresis donors must undergo insertion of a relatively large-bore intravenous catheter to allow for processing of the large volumes of blood that are needed to collect an adequate number of platelets. Plateletpheresis collection techniques have been refined such that approximately 3×10^{11} platelets—that is, approximately the same number of platelets contained in a 6-pack of pooled-donor platelets—usually can be collected from a single donor in a single session. Modern apheresis devices are equipped with software that can predict the platelet yield based on the donor's size, platelet count, and hematocrit.

As with RBCs, leukoreduction of platelet products can result in reductions in 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 overgrowth with prolonged storage. Bacteriologic considerations limit the storage of platelets to 5 days. Clinical studies indicate that there is relatively little loss of platelet function and viability as long as storage is limited to approximately 5 days. 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. A recent study in a murine platelet transfusion

model suggests that cycling platelets between cold (4°C for 12 hours) and warm (37°C for 30 minutes) temperatures may extend the viable shelf life of platelets without compromising *in vivo* recovery or function.

Clinical transfusion of platelets

Clinical case

A 56-year-old multiparous female develops acute myeloid leukemia and is admitted to the hospital for intensive induction therapy. The platelet count rapidly decreases to <10,000/µL, and she responds well initially to prophylactic transfusion with pooled platelet concentrates. During the second week of hospitalization, however, her 1-hour postinfusion platelet count increments are persistently <5,000/µL. Having obtained HLA typing on the patient before initiating the induction therapy, the attending physician asks the blood bank director to obtain HLA-matched platelets from the regional blood center.

Prophylactic platelet transfusion

The most appropriate threshold for prophylactic platelet transfusion in thrombocytopenic patients remains difficult to determine. Early studies suggested that significant spontaneous bleeding does not occur until the plt count is 5,000 plts/µL or less. For many years, a platelet count of ≤20,000/µL was considered to be the appropriate threshold for prophylactic platelet transfusion. Several prospective randomized transfusion trials, however, showed no differences in hemorrhagic risks between a prophylactic platelet transfusion triggers of ≤10,000 and ≤20,000/µL. A broad variability in practice exists in which some centers administer prophylactic platelet transfusions only if the count decreases <5,000/µL, whereas other centers have maintained thresholds in the 10,000-20,000/µL range. A preliminary report from the TOPS trial (conducted in the United Kingdom and Australia) did not support noninferiority of transfusing platelets for bleeding only compared with a standard prophylactic transfusion approach in patients undergoing leukemia induction or HSCT. Common indications for raising the prophylactic platelet transfusion target include blast crisis or acute promyelocytic leukemia during induction; recent or upcoming invasive procedures; qualitative platelet dysfunction due to uremia, drugs, or other causes; concurrent coagulopathy; anemia; fever; hypertension; and acute pulmonary processes, all of which are thought to increase the risk of spontaneous bleeding. In patients with 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. More realistic target counts should be set in patients who do not respond well to platelet transfusion, such as

those with splenomegaly and immune-mediated platelet transfusion refractoriness.

The survival of endogenously produced platelets in healthy individuals is in the range of 7 to 9 days. The half-life of platelets in individuals undergoing induction chemotherapy for acute leukemia, HSCT, or other acute medical situations, however, is typically 1-3 days or less. Most centers check the platelet count in patients receiving platelet transfusion support at least every 24 hours. Checking an immediate (<1 hour) postinfusion platelet count, however, is often extremely helpful in monitoring the platelet response and half-life. In addition, as detailed later, poor immediate postinfusion counts may reflect the presence of alloantibodies to HLA antigens, which is an indication for requesting HLA-matched products. Table 12-2 summarizes the major platelet preparations and their respective indications.

Choice of platelet product

The current evidence does not support the previously held notion of superiority of apheresis platelets over pooled platelets. 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 infections. Because of the very low absolute magnitude of the infectious risk associated with transfusion of blood products (discussed later in this chapter) in comparison with the magnitude of the other treatment-related and disease-related risks to which most platelet transfusion recipients are subject, the cost effectiveness of requiring single-donor transfusions for all platelet transfusion recipients has been seriously questioned.

In contrast to the availability of universal RBC donors (O negative) and universal plasma donors (AB-positive or negative), universal donors for platelets do not exist because platelet products contain substantial quantities of plasma (typically 200-400 mL) and a small volume of RBCs expressing both ABO and RhD antigens, in addition to platelets expressing ABO antigens. For example, group O platelets contain anti-A and anti-B isohemagglutinins that would react against the RBCs of all but type O recipients, whereas group AB platelets likely will yield a decreased platelet count increment when transfused into all but type AB recipients. Ideally, patients should receive ABO-identical platelets; in reality, platelets are in short supply because of their shelf life (5 days) and may be issued to patients without regard for ABO compatibility except in the case of neonates. Recent data suggest that platelets obtained from group A₂ donors—that is, individuals who are blood type A but have substantially weaker than average A antigen expression on their RBCs—can be administered to group O recipients without the reduction in platelet count increment that ordinarily would be expected in the A-into-O platelet transfusion setting. Transfusion of a platelet product

from an RhD-positive donor to an RhD-negative recipient uncommonly 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 that one administers to a thrombocytopenic patient depends on the therapeutic goal. If, for example, the primary goal is to administer a sufficient number of platelets to prevent bleeding in a myelosuppressed patient, the target would be to keep the trough peripheral blood platelet count above $\sim 10,000/\mu\text{L}$. To select the appropriate platelet dose, one must consider factors that affect the response to individual transfusions, including the size of the patient and the presence of splenomegaly, active bleeding, disseminated intravascular coagulation, platelet antibodies, and other factors. In addition, it appears that thrombocytopenia itself decreases platelet survival such that more severely thrombocytopenic patients may require higher doses or more frequent transfusions to maintain a particular peripheral blood platelet level.

The U.S. Food and Drug Administration (FDA) guidelines dictate that SDPs 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 starting dose, and it is expected to increase the platelet count by 20,000–30,000/ μL . On average, this dose can be supplied by a single apheresis unit of SDP or a pooled-donor 5- or 6-pack. If a patient is being managed as an outpatient, larger doses of platelets may extend the interval between transfusions. A multicenter randomized trial comparing low-, average-, and high-platelet dosing transfused prophylactically for a platelet count of $10,000/\mu\text{L}$ in patients undergoing chemotherapy or HSCT (where the average is equivalent to 4- to 6-pooled platelet concentrates or 1 apheresis unit of SDP) found that the low-dose group did not experience increased bleeding and 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 two consecutive postinfusion platelet count

increments $\leq 10,000/\mu\text{L}$. A more formal definition of refractoriness, which adjusts for both the size of the patient and the number of platelets actually infused, uses the so-called *corrected count increment* (commonly referred to as the CCI), which is based on a platelet count obtained within 1 hour of transfusion calculated as follows:

$$\text{CCI} = \text{Body Surface Area (BSA; m}^2\text{)} \times \text{Platelet Count Increment} \times 10^{11}/\text{Number of Platelets Transfused}$$

For example, if 3×10^{11} platelets are transfused to a patient with a BSA of 1.8 m^2 , and the posttransfusion increase in platelet count is $23,000/\mu\text{L}$, then the CCI = $1.8 \text{ m}^2 \times 23,000/\mu\text{L} \times 10^{11}/3 \times 10^{11} = 13,800$. Platelet transfusion refractoriness often is defined as two or more consecutive postinfusion CCIs of $< 5,000$ – $7,500$.

As noted, alloimmunization to HLA antigens accounts for a large fraction of cases of platelet transfusion refractoriness. In larger patients, simply increasing the platelet dose is often sufficient to achieve adequate postinfusion increments; a trial of fresh ABO-matched platelets may be worthwhile as well. In the absence of obvious nonimmune causes of platelet transfusion refractoriness, such as marked splenomegaly, disseminated intravascular coagulation, or the use of relatively small platelet doses in relatively large patients, the clinician should request an anti-HLA antibody evaluation. Anti-HLA antibodies, and their specificities, typically are detected on high-throughput platforms such as Luminex microbeads coated with HLA class I and II antigens. In patients whose panel-reactive antibody (PRA) screen is positive, one should select platelets based on HLA matching, avoiding the antibody specificities found in the patient, or platelet cross-matching, although these methods do not always 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 appears to resolve spontaneously; thus, the requirement for HLA-matched products may not persist indefinitely.

Normally, platelets express HLA-A and HLA-B antigens, but not HLA-C, -DR, -DQ, or -DP antigens. Therefore, most blood centers attempt to optimize matching only at the HLA-A and HLA-B loci. Depending on the HLA type of an individual, one may have little or great difficulty in locating platelets that are HLA identical, or at least within the same CREG as the patient. An A, B, C, D grading system has been developed to semiquantitatively define the degree to which the platelet donor and the platelet recipient are matched at these loci, although the predictive value of this system is modest.

The relatively low-stringency, serologic, four-loci, HLA-matching protocols that transfusion medicine specialists

typically use to select platelet products is quite different from the relatively high-stringency, molecular-level, 10- to 12-loci, HLA-matching schemas that bone marrow transplantation (BMT) specialists typically use to select HSCT donors. Nevertheless, for some patients with unusual HLA types, such as patients who are members of certain ethnic groups, locating an appropriate HLA-matched platelet donor may still be difficult. Relying solely on HLA matching has certain shortcomings. In some cases, it is overly restrictive because some HLA-B locus antigens are not present on platelets. In addition, there is only a modest correlation between the degree of HLA match and the observed postinfusion platelet count increment. For these reasons, there has been a great deal of interest in adopting a platelet cross-matching approach similar to that used in RBC compatibility testing. In the latter approach, a sample of the patient's serum is incubated with two or more small aliquots of platelets from candidate donor units, and those units that manifest the least cross-reactivity are selected for transfusion. At present, it is not clear which method (HLA matching or platelet cross-matching) 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 pass 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 often can be overcome by obtaining platelets from the original stem cell donor. In other settings, therapeutic modalities used have included corticosteroids, plasmapheresis, intravenous γ -globulin, frequent platelet transfusion, continuous-infusion platelet transfusion, and ϵ -aminocaproic acid. Few randomized clinical data support any one of these modalities over the others. As noted, realistic targets 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 discernable benefit. In addition, it may result in reduced availability of platelet products for other patients when supplies are limited.

Key points

- Antibodies directed against HLAs commonly develop following blood transfusion or pregnancy and are the most important cause of immune platelet transfusion refractoriness.

Key points (continued)

- Human platelet alloantigens are polymorphisms on platelet surface glycoproteins that may also mediate platelet transfusion refractoriness, as well as NAIT, PTP, and alloimmune thrombocytopenia following HSCT.
- Prophylactic platelet transfusion should be considered when the peripheral blood platelet count decreases below approximately 10,000/ μ L, but the platelet count target should be increased in the presence of specific risk factors for bleeding, including recent or upcoming invasive procedures, coagulopathy, fever, hypertension, or acute pulmonary processes.

Granulocyte transfusion

Granulocyte antigen systems

As is the case with RBC antigens and platelet antigens, antigen systems on neutrophils can be classified as shared or restricted. Shared antigens, which also are found on other hematopoietic cells, include the ABO, HLA, and Ii systems. The biallelic NA-1/NA-2 antigen system, now called HNA-1a/HNA-1b, appears to be the most antigenic, and donor-recipient or fetal-maternal mismatches involving the HNA-1a/HNA-1b system appear to be responsible for a significant percentage of reported cases of neonatal alloimmune neutropenia (NAIN), granulocyte transfusion refractoriness, transfusion-related acute lung injury (TRALI; see discussion later in the chapter), and delayed neutrophil recovery or secondary graft failure following HSCT.

The molecular characterization of neutrophil-restricted antigens is incomplete, although some of the more important antigen systems, such as the HNA-1a/HNA-1b system, now have been characterized. Common properties of neutrophil-specific alloantigen systems include their absence on early myeloid precursors and the acquisition of expression during neutrophil differentiation. NA antigens reside on a neutrophil IgG Fc receptor called Fc γ RIII, also known as CD16. Fc γ RIII is linked to the outer leaflet of the cell membrane bilayer by a glycosylphosphatidylinositol (GPI) anchor. As a result, NA 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. An antibody to the Fc γ RIII receptor has been identified as a cause of neonatal granulocytopenia in cases in which the mother was congenitally deficient in this protein and the fetus expressed the protein through paternally derived genes.

Alloantibodies to neutrophil-specific antigens appear to play an important role in some cases of febrile transfusion reaction and TRALI. In one study, more than one-third of

patients undergoing HSCT acquired antibodies directed against neutrophils in the posttransplantation period and the presence of such antibodies was independently correlated with both delayed neutrophil engraftment and postengraftment neutropenia—that is, secondary graft failure. 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 has been used to induce neutrophilia in donors, and this slightly increases the granulocyte yield. Pretreatment of granulocyte donors with small doses of 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, but the duration of the follow-up period in such studies is somewhat limited. Because of the short natural half-life of granulocytes and a 24-hour expiration period, granulocytes should be harvested, transported, and infused into the intended recipient within a matter of hours. This often is confounded by current constraints related to the time required for infectious disease screening of the donor, particularly with nucleic acid testing (NAT) for HIV, hepatitis C and B viruses, and West Nile virus (WNV), which can take 24–48 hours to complete. Because of these issues, some institutions have procedures in place by which the physician of the intended granulocyte recipient can transfuse 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 typically are selected from pedigreed apheresis platelet donors who have had documented negative infectious disease testing within the prior few months, 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 the transfusion of granulocytes. The initial

treatment of patients with neutropenic fever should consist of broad-spectrum antibiotics and recombinant growth factors. Granulocyte transfusions should be considered only in patients with ongoing neutropenia in whom bacterial or fungal infections persist or progress despite the administration of appropriate antibiotics and antifungal agents. Other patients who may benefit from granulocyte transfusions include patients with neutropenic sepsis caused by organisms resistant to antibiotics. An RCT is ongoing in the United States comparing conventional therapy with the addition of G-CSF–mobilized granulocytes (see the section “Transfusion Support after HSCT”) in HSCT patients with neutropenic fever.

Once the decision to use granulocyte transfusions has been made, an adequate dose should be given. A minimum dose of $2\text{--}3 \times 10^{10}$ 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 should be used unless effective RBC sedimentation is performed. Daily granulocyte transfusions are continued 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, intervenes. 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 by a few hours because anecdotal evidence suggests that pulmonary toxicity otherwise is increased. Serologic testing for antineutrophil antibodies is not performed routinely, but it is indicated if significant transfusion reactions develop. If antibodies are found, leukocytes from compatible donors probably should be used, particularly the peripheral blood stem cell (PBSC) donor, if applicable. Leukocyte reduction filters obviously should not be used with granulocytes. Unlike stem cells and donor lymphocyte infusions (DLIs), however, granulocytes should undergo γ -irradiation. In addition, if the potential for CMV transmission is a concern, then granulocytes collected from CMV-seronegative donors should be used because leukoreduction filters cannot be used.

Key points

- Antibodies directed against HNA-1a/HNA-1b and other neutrophil antigen systems may mediate TRALI, refractoriness to granulocyte transfusions, NAIN, alloimmune neutropenia following HSCT, and qualitative neutrophil dysfunction.
- Transfusion of granulocytes should be considered in patients with severe prolonged neutropenia and antibiotic-refractory infections.

Transfusion of plasma products

Plasma

As is the case with packed RBCs, units of plasma most commonly are obtained from units of whole blood donated by volunteer blood donors. The traditional nomenclature of FFP applies to plasma frozen within 8 hours of collection and used within 24 hours of thawing. Other types of plasma commonly used 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 a total of up to 5 days after thawing. A standing inventory of thawed plasma can be made available much more quickly in emergency bleeding situations. New viral inactivation methods to sterilize plasma exist but are not yet FDA 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 use of ultraviolet-activated psoralen derivatives.

In theory, plasma can be used to treat acquired or congenital deficiencies of virtually any circulating procoagulant or anticoagulant factor. It is standard practice, however, to use relatively purified pharmaceutical preparations of coagulation-related proteins when available, including factors VII, VIII, IX, protein C, and antithrombin. Thus, the most common indications for plasma include situations in which multiple factor deficiencies are present simultaneously, such as patients with liver disease, with disseminated intravascular coagulation, on warfarin, with vitamin K deficiency requiring urgent therapy, with dilutional coagulopathy of massive transfusion secondary to acute blood loss, or requiring plasma exchange for such indications as thrombotic thrombocytopenic purpura (TTP). These products (FFP, PF24, and thawed plasma) often are used interchangeably; however, because of the decrease in levels of the 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. For hemophilia A, optimal management is with factor VIII concentrate, or cryoprecipitate if the factor concentrate is not available, whereas plasma, preferably FFP, should be used to manage factor V deficiency. ADAMTS13 (a disintegrin and metalloprotease with thrombospondin) protease activity is stable up to 5 days of storage and, as such, thawed plasma can be used for plasma exchange in TTP.

The ratio of plasma to RBC transfusion is relatively high in the United States. Most prophylactic plasma transfusions to correct mild prolongations of coagulation values before an invasive procedure do not actually correct the

result and often are not indicated. RCTs to determine the appropriate indications and dosing of plasma therapy are sorely needed. In emergency situations involving life-threatening bleeding in patients with hemophilia A or B in cases in which factor concentrates are not readily available, it is theoretically possible to initiate replacement therapy with FFP (for factor IX) or cryoprecipitate (for factor VIII, see the section "Cryoprecipitate"), but adequate factor correction (eg, from 0%-50% normal) would require a substantial, often prohibitive, volume of plasma. 4-Factor prothrombin complex concentrates with adequate FVII content (in addition to prothrombin, FIX, and FX) increasingly are being used to reverse warfarin-related bleeding, although these newer concentrates are not yet licensed for use in the United States.

Cryoprecipitate

Cryoprecipitate is prepared by thawing FFP at 4°C and then removing the supernatant from the cryoprecipitable proteins following centrifugation at 1°C-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 proteins S. Cryoprecipitate alone is not the optimal replacement strategy in patients with disease processes that deplete both procoagulants and anticoagulants, such as disseminated intravascular coagulation or severe hepatic failure. The most common clinical indication for the transfusion of cryoprecipitate is congenital or acquired hypofibrinogenemia. In theory, cryoprecipitate can be used to treat von Willebrand disease or hemophilia A, when virally inactivated factor concentrates, which are preferable, are not available. Cryoprecipitate also has been used to treat qualitative platelet dysfunction because of uremia, and life-threatening hemorrhage secondary to thrombolytic therapy. The supernatant plasma (sometimes referred to as *cryo-supernatant* 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. Unlike intravenous immunoglobulin (see the next section), factor concentrates, and albumin, cryoprecipitate is not pathogen inactivated, and a pool of 8-10 units of cryoprecipitate needed to correct hypofibrinogenemia in an adult carries a multiplicative donor exposure risk compared with a single unit of RBC transfusion. Fibrinogen concentrate that has undergone viral inactivation is available in some countries but is not yet widely available in the United States.

Immunoglobulin

Commercially available intravenous immunoglobulin (IVIg) products typically are 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, hepatitis C, or HIV appears to be negligible, although concerns remain regarding the potential transmission of certain difficult-to-inactivate pathogens, such as parvovirus B19 and the agent responsible for transmitting Creutzfeldt-Jakob disease. 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) appears 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 or multiple myeloma. In autoimmune cytopenias such as ITP, IVIg often is considered to be the best emergency intervention when a rapid, albeit often transient, response is required.

The mechanism by which IVIg ameliorates autoantibody destruction of blood cells is unknown. Historically, it has been assumed that the infused IgG blocked Fc receptors on phagocytic cells of the reticuloendothelial system. Numerous other theories have been proposed, including autoantibody neutralization by anti-idiotypic antibodies, cytokine modulation, and complement neutralization. None of these theories have been validated in controlled experimental studies. Recent studies have provided experimental evidence that IVIg may serve to create soluble immune complexes that interact with activating Fcγ-receptor on dendritic cells, which leads to the inhibition of macrophage phagocytic activity. Recent studies in murine models of ITP have demonstrated that the immunomodulatory effects of IVIg can be reproduced by the adoptive transfer of CD11c-positive dendritic cells that have been primed with IVIg.

A significant proportion of patients receiving IVIg develop a positive direct antiglobulin test (DAT, also known as a direct Coombs test) because of the presence of anti-A or anti-B derived from type O individuals in the donor pools, and there are occasional case reports of overt acute alloimmune hemolytic anemia developing. Fever is a relatively common sequelae of IVIg administration and does not necessarily preclude the administration of additional IVIg.

Key points

- The most common indications for the transfusion of plasma include rapid reversal of anticoagulant effects; treatment of deficiencies of coagulation factors for which specific coagulation replacement products are not available; treatment of multiple coagulation factor deficiencies in conditions, such as disseminated intravascular coagulation or vitamin K deficiency; and plasma exchange in patients with TTP.
- The most common indication for transfusion of cryoprecipitate is hypofibrinogenemia.

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. Although a detailed description of such testing is beyond the scope of this chapter, it is nevertheless 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. For more in-depth reading, clinicians are encouraged to consult several excellent transfusion medicine texts listed in this chapter's bibliography, particularly the *Technical Manual* published by the American Association of Blood Banks.

ABO/Rh(D) typing

Determining a patient's ABO blood group includes two independent sets of tests that should yield complementary results (Table 12-3). 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. This test exploits the phenomenon noted earlier in which individuals naturally produce the isoagglutinins to the A or B antigens that their RBCs lack. The patient's serum is mixed with reagent RBCs that are known to be either blood group A or B and agglutination is assessed. Table 12-3 illustrates the expected forward- and reverse-typing results for the four possible ABO blood types.

Discrepancies between the forward- and reverse-typing reactions occur and sometimes can be explained by examining the patient's recent transfusion history. For example, a blood group B individual given type O RBCs in an emergency situation could continue to demonstrate only the appropriate anti-A isoagglutinin by reverse typing but

Table 12-3 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:	
	Anti-A	Anti-B	A1 RBC	B RBC
O	0	0	+	+
A	+	0	0	+
B	0	+	+	0
AB	+	+	0	0

Reactivity typically is graded on a scale from 0 (nonreactive) to + (strongly reactive).

RBC = red blood cell.

also could show a mixed field of RBCs, that is, both agglutinated (the patient's blood group B cells) and unagglutinated RBCs (the transfused blood group O cells) upon forward typing with anti-B. In the case of newborns, forward- and reverse-typing discrepancies can be expected to occur because the production of the appropriate isoantibodies is delayed for several months while their immune systems are maturing. Thus, the ABO type for all newborns would be interpreted as blood group AB if determined based solely on their reverse-typing reactions. For this reason, only forward typing is performed on newborns. Other settings for forward- and reverse-typing discrepancies include patients who have undergone ABO-mismatched HPSCs, particularly during their transition from one ABO blood type to another. Whatever the cause, it is important to resolve the etiology for the ABO typing discrepancy to select the appropriate ABO type for transfusion.

Typing for the presence or absence of the Rh(D) antigen on RBCs is an important part of determining a patient's blood type. Typing for D does not involve a reverse typing as for the ABO typing process because anti-D is not constitutively expressed in the sera of Rh-negative individuals. The issue of weak D versus partial D phenotypes and their clinical significance previously has been discussed in the section on Rh antigens.

Antibody screen and specificity identification

In general, a patient who never has been transfused or pregnant would be expected to have only the appropriate naturally occurring isoantibodies based on his or her ABO type. For such patients, transfusion with blood products that are selected solely on the basis of ABO compatibility should, in theory, be well tolerated. Because patient transfusion and pregnancy histories can be unreliable or not readily obtained, however, and possible exposure and hence sensitization to non-ABO blood group antigens cannot be ruled out with

absolute certainty, it is the standard of care to test all patient sera for the possible 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.

Alloantibody exclusion and identification are the most time-consuming of the pretransfusion tests. Antibody screening consists of performing agglutination reactions between patient sera and 2-3 reagent RBCs whose extended phenotype (ie, antigenic composition across many blood groups) is known and includes all common, clinically significant alloantigens. If the patient's serum does not react with the screening cells, then the patient is said to have a *negative antibody screen* and ABO-compatible units can be selected for cross-match, as described in the following section.

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 run against a larger set of reagent red cells (typically 11-16, 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 cells, the alloantibody specificities can be identified to an acceptable degree of certainty. All reagent RBCs used as screening cells and panel cells are selected purposely to be blood group O so that the presence of anti-A or anti-B isoantibodies will not affect the results.

On the basis of the results of the antibody identification panel, units of ABO/Rh-compatible RBCs are selected from inventory, and RBCs from attached segments from each of the units are tested with preparations of reagent antisera to identify antigen-negative units.

Although agglutination reactions are the end point of all currently available methods for antibody screening, various test methods are available, including tube testing, gel-based testing, and solid phase testing, among others. Differences in the sensitivity, specificity, interfering substances and detection of clinically insignificant antibodies exist among the available methods. For example, gel and solid phase methods are formulated specifically to identify IgG antibodies and not detect IgM antibodies, which typically are insignificant for transfusion purposes but may be important to identify in patients with suspected AIHA, for example. Therefore, if the purpose of testing is to evaluate for the presence of a cold agglutinin or other IgM antibody, then consultation with a blood bank may be required to ensure that the test method that was used does not ignore such antibodies. The antibody screen, indirect antiglobulin test (IAT) and indirect Coombs test are all different names for the same test; in other words, if the patient has an available antibody screen result, there is no added utility to ordering a separate IAT or indirect Coombs test.

The antibody screen 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 by virtue of the presence of panreactive serum antibodies (ie, anti-RBC antibodies that not only bind to their own RBCs but also to all RBCs, including reagent screening and panel cells). As a result, the presence of additional alloantibodies to specific blood group alloantigens may be masked by the autoantibodies. Time-consuming methods known as absorption techniques must be used in these cases and are discussed in the section on AIHA.

Cross-matching

There are two basic types of cross-match procedures, and the one used depends on the results of the patient's antibody screen. If the screen is negative and the blood bank has historical records indicating no alloantibodies in the patient, then an immediate spin cross-match may be carried out in which the patient's serum is mixed at room temperature with an aliquot of RBCs from the prospective ABO-compatible unit and the absence of agglutination due to IgM isohemagglutinins is verified. The purpose of this type of cross-match is to serve as a final check for ABO compatibility. It is now acceptable practice, however, for blood banks to perform an electronic or computer cross-match, 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. This type of cross-match is thus a *virtual cross-match* because no physical mixing of cells and sera takes place.

The other type of cross-match procedure is known as a full or Coombs cross-match. This type of cross-match is required for cases in which the patient has a historical or currently positive antibody screen. After identifying prospective ABO/Rh-compatible units that are negative for the antigen(s) against which the patient has alloantibody(ies), a full cross-match is performed. This consists of incubating patient serum with RBCs from the selected RBC units and carrying the testing through from the immediate spin to the Coombs (IgG or antihuman globulin) phase to ensure that the selected units are not only ABO compatible but also are compatible at the IgG phase. This additionally ensures that the units lack the additional antigens for which the patient's serum contains preformed alloantibodies.

Incompatible cross-matches may be seen in a number of situations, most commonly in the presence of warm autoantibodies or panagglutinins. Understanding the reason for the incompatible cross-match is critical to determining the risk versus the benefit of proceeding with transfusion of a cross-match-incompatible RBC unit. Consultation with a blood bank physician often is warranted in these situations.

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 serum must be screened for the presence of red cell alloantibodies that may have formed following a previous transfusion or through pregnancy.
- If a patient's serum lacks clinically significant red cell alloantibodies, then an immediate spin or computer cross-match is performed with prospective RBC units to ensure ABO blood group compatibility.
- If a patient's serum demonstrates the presence of clinically significant red cell alloantibodies, then ABO/Rh-compatible RBCs must be identified that lack the corresponding antigen(s). These prospective units must undergo a full or Coombs cross-match to ensure that the selected units are not only ABO compatible but also lack the additional antigens to which the patient's serum contains preformed alloantibodies.

Apheresis

Plasma exchange and plasmapheresis

A number of the more common indications for therapeutic apheresis are given in Table 12-4. For a more comprehensive list and discussion using an evidence-based medicine approach, a recent special issue of the *Journal of Clinical Apheresis* is cited in this chapter's bibliography. Plasma exchange traditionally has involved centrifugation of whole blood removed from the patient. Typically, one plasma volume of patient plasma is removed and replaced with 5% albumin (or FFP, in the case of TTP), which is combined with the patient's cellular blood elements in the extracorporeal circuit, and returned to the patient. Centrifugal apheresis typically is performed in a continuous-flow fashion, so that the patient remains euvolemic throughout the procedure. An alternative technology, namely, hemofiltration, more recently has been introduced and involves the extraction of selected plasma constituents by passing the patient's blood over specially designed membranes. With this form of ultrafiltration, greater selectivity is possible so that clotting factors and albumin may be retained by the patient. This technology is designed primarily to remove high-molecular weight immune complexes or low-density lipoprotein (LDL). Nevertheless, conventional centrifugal plasma exchange continues to be used far more frequently than hemofiltration for the previously listed indications in the United States. A course of therapy typically consists of 5-7 alternate-day sessions using colloid replacement, except in the case of TTP in which case daily exchanges with FFP or thawed plasma are performed until the platelet count normalizes. The adverse effects of plasma exchange are driven by complications related to the central venous catheter, if

Table 12-4 Abbreviated list of therapeutic apheresis procedures grouped by ASFA indication category.

Disease/disorder	Procedure
<i>Category 1. Standard and acceptable therapy, including primary therapy.</i>	
Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)	Plasmapheresis
Cryoglobulinemia	Plasmapheresis
Cutaneous T-cell lymphoma; mycosis fungoides (erythrodermic)	Extracorporeal photopheresis
Familial hypercholesterolemia (homozygotes)	Selective absorption
Goodpasture syndrome	Plasmapheresis
Guillain-Barré syndrome	Plasmapheresis
Hyperleukocytosis/leukostasis	Leukapheresis
Hyperviscosity in monoclonal gammopathies	Plasmapheresis
Myasthenia gravis	Plasmapheresis
Sickle cell disease (life and organ threatening)	Red blood cell exchange
TPP	Plasmapheresis
ANCA-associated rapidly progressive glomerulonephritis	Plasmapheresis
Babesiosis, severe	Red blood cell exchange
Antibody-mediated renal transplant rejection	Plasmapheresis
<i>Category 2. Sufficient evidence to suggest efficacy; acceptable therapy on an adjunctive basis.</i>	
ABO-incompatible hemopoietic progenitor cell transplantation	Plasmapheresis
Cold agglutinin disease, life-threatening	Plasmapheresis
Familial hypercholesterolemia	Plasmapheresis
Familial hypercholesterolemia (heterozygotes)	Selective absorption
Graft-versus-host disease (skin)	Extracorporeal photopheresis
Catastrophic antiphospholipid syndrome	Plasmapheresis
Malaria	Red blood cell exchange
Sickle cell disease (stroke prophylaxis)	Red blood cell exchange
<i>Category 3. Insufficient evidence for efficacy; uncertain benefit-to-risk ratio. Conditions that may fall into this category might be an exception effort for an individual patient.</i>	
Aplastic anemia; pure red blood cell aplasia	Plasmapheresis
Coagulation factor inhibitors	Plasmapheresis/immunoabsorption
Graft-versus-host disease (nonskin)	Extracorporeal photopheresis
Hyperleukocytosis/leukostasis (prophylaxis)	Leukapheresis
Myeloma and acute renal failure	Plasmapheresis
Posttransfusion purpura	Plasmapheresis
Warm- and cold-type autoimmune hemolytic anemia	Plasmapheresis
<i>Category 4. Disorders for which controlled trials have not shown benefit or anecdotal reports have been discouraging.</i>	
Diarrhea-associated HUS	Plasmapheresis
Dermatomyositis/polymyositis	Plasmapheresis
ITP (refractory)	Plasmapheresis
Rheumatoid arthritis	Plasmapheresis
SLE nephritis	Plasmapheresis

ANCA = antineutrophil cytoplasmic antibody; ASFA = American Society for Apheresis; HUS = hemolytic-uremic syndrome; ITP = immune thrombocytopenic purpura; SLE = systemic lupus erythematosus; TPP = thrombotic thrombocytopenic purpura.

needed (infection, bleeding, thrombosis), and the risk of reactions with plasma replacement in TPP.

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, irradiating the mononuclear cells with ultraviolet A irradiation, and returning the irradiated mononuclear cells to the patient; the whole process takes about 2.5 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 4-6 months. Response correlates with the presence of circulating clonal tumor cells and a CD8-mediated antitumor response. ECP also is used to treat chronic GVHD after allogeneic stem cell transplantation and has a 70% response rate when used as second-line therapy.

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 uses a dextran sulfate column to bind LDL and very

low-density lipoprotein (but sparing high-density lipoprotein [HDL]). Heparinized blood separated into plasma by hollow fiber filters perfuses twin columns alternately, each column being regenerated automatically between successive adsorption cycles. A typical procedure reduces LDL by 50%-75% and is performed every 2 weeks; fibrinogen is lowered by 20%-40%, although bleeding or other complications are rare. Some authorities believe that homozygous children should start LDL apheresis around age 7 years to prevent premature atherosclerosis. The procedure avoids the non-specific removal of immunoglobulins as well as HDL cholesterol in standard plasmapheresis.

Exchange transfusion

RBC exchange transfusion therapy is performed most often in patients with sickle cell disease to treat acute complications of the disease, such as central nervous system infarction and acute chest syndrome. Preoperative exchange transfusion is indicated when the surgeon intends to create a dry field using a vaso-occlusion tourniquet (common in orthopedic procedures) or if the procedure requires a long anesthesia time. In addition, exchange transfusion is indicated to prevent strokes in sickle cell patients who have had an ischemic stroke in the past, or in children at high risk for development of stroke. In such circumstances, patients should be transfused with blood that is known to be negative for hemoglobin S; using directed-donor or family-donor blood that has not been subjected to hemoglobin electrophoresis, or sickle screening, may impart a risk of infusing additional hemoglobin S, possibly decreasing the potential therapeutic benefit. Most clinicians attempt to achieve a final concentration of hemoglobin S of $\leq 30\%$. In many centers, an apheresis machine is used to perform RBC exchange; the patient's erythrocytes are removed and replaced with donor erythrocytes while the patient's own plasma is being returned continually, thus inducing minimal disturbance of hemodynamic and coagulation parameters. In other centers, incremental phlebotomy followed by infusion of donor RBCs is performed without the use of hemapheresis, particularly in small children who do not have the required circulatory volume to be exchanged using an apheresis machine. When manual exchange is done, careful attention should be paid to the potential for volume depletion. The goal of exchange transfusion in most situations such as acute chest syndrome and stroke, whether performed manually or via automated RBC apheresis, is to achieve a hematocrit of 30% with a hemoglobin S of $\leq 30\%$.

PBSC harvesting

Mobilization refers to the technique of increasing the number of circulating progenitor cells in the peripheral blood. 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, or cleaves, adhesion molecules on the surface of HSCs, progenitor cells, precursor cells, and mature neutrophils and mobilizes clinically significant numbers of HSC into the peripheral blood. Many mobilization regimens combine chemotherapy with growth factors. Although earlier studies suggested that leukapheresis should commence when the white cell count reaches $1 \times 10^9/L$, more recent data suggest that a more optimal time to start collecting is when the white cell count exceeds $10 \times 10^9/L$. Cyclophosphamide (at 2.5-4.0 g/m²) followed by G-CSF at 5-10 µg/kg/d is among the more commonly used protocols. The white cell count reaches $1-10 \times 10^9/L$ around day 11-13 after chemotherapy. Leukapheresis usually is scheduled for day 10-12 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 febrile neutropenia with chemotherapy and can be used in allogeneic donors. G-CSF at 10 µg/kg/d has been the mobilization regimen of choice for allogeneic PBSC donors. With this regimen, leukapheresis begins on day 5, when the white cell count is $20-50 \times 10^9/L$. 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. For instance, for a target collection of $2 \times 10^6/kg$ CD34+ cells, the preceding day CD34+ cell count in the peripheral blood should exceed $20 \times 10^6/L$. If collections are planned to take place over a number of consecutive days, many centers begin collections when the peripheral CD34+ cell count is lower (eg, $10 \times 10^6/L$).

Although the administration of mobilizing doses of G-CSF can induce seemingly worrisome degrees of leukocytosis—transient peripheral blood leukocyte counts of 80,000/µL 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 for PBSC mobilization is splenic rupture; at least four cases of splenic rupture have been reported in healthy adult PBSC donors, most commonly after 5 days of daily G-CSF administration.

Large-volume leukapheresis (LVL) refers to the processing of large volumes of blood (15-30 L over 5 hours) made possible by current automated cell separators; 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 two techniques can be combined for maximal benefit.

The principles of LVL involve establishing good venous access that would permit flow rates on the order of 80–110 mL/min. This may necessitate the insertion of an apheresis catheter in a central vein. 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. Newer cell separators may be able to collect PBSCs with less platelet loss.

A relatively common problem with PBSC harvesting is inadequate collection. Although multiple definitions of inadequate collection have been used, it is clear that the incidence of inadequate collection is much higher in heavily pretreated patients than in healthy donors. In healthy PBSC donors, age, white ethnicity, and female sex were associated with lower post-G-CSF peripheral blood CD34⁺ counts, which correlate with 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 HSC 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 as a single agent is being studied in healthy PBSC donors. The adverse effect profile of plerixafor (mostly gastrointestinal) does not appear to overlap with that of G-CSF.

Key points

- The most common hematologic conditions for which therapeutic apheresis is indicated include paraproteins causing hyperviscosity syndromes, TTP, and exchange transfusion in patients with sickle cell disease in whom acute complications develop or prolonged surgery is anticipated.
- A variety of nonhematologic antibody-mediated autoimmune disorders can be successfully treated with apheresis, including Goodpasture syndrome, Guillain-Barré syndrome, and myasthenia gravis.

Transfusion support in special clinical settings

Patients with aplastic anemia and other candidates for HSCT

As autologous and nonmyeloablative HSCTs are being offered to a wider population of patients with hematologic

malignancies, the clinician must take into account the possibility that many patients newly diagnosed with hematopoietic malignancies are likely to become potential candidates for HSCT at some point in their clinical course. Therefore, it is important to avoid administering transfusion products obtained from family members because they may increase the risk of graft rejection via alloimmunization to minor histocompatibility antigens. For newly diagnosed patients with acute leukemia, it is useful to perform HLA typing earlier in the course of induction therapy and to anticipate problems in platelet support in patients who are at risk of HLA alloimmunization, such as multiparous females. HLA typing results obtained upfront will be useful for potential allogeneic HSCT.

Transfusion support in patients with aplastic anemia merits discussion because several studies have supported a strong association between the number of blood product exposures before allogeneic HSCT and the risk of the often-fatal complication of graft rejection in such patients. It is believed that recipient-derived T-cells directed against a variety of hematopoietic targets contribute to the genesis of the aplastic anemia itself and also may be responsible for the particularly high incidence of graft rejection that has been observed among patients undergoing HSCT for aplastic anemia. Administration of irradiated, leukoreduced blood products in such patients may reduce the risk of subsequent graft rejection. Serious attention must be paid to minimizing the number of blood product exposures in patients with aplastic anemia because older patients are often eligible for HSCT with nonmyeloablative conditioning. Menstrual suppression in young women can be helpful. RBCs should be transfused only for significantly symptomatic anemia. Single-donor (apheresis-derived) platelet products should be used in preference to pooled random-donor platelets whenever possible; antifibrinolytics are a useful adjunct to a lower platelet transfusion threshold.

Hematopoietic stem cell infusion

In the setting of allogeneic HSCT, PBSCs typically are infused “fresh,” without cryopreservation. RBC or plasma depletion of the PBSC component may be required if the donor is major or minor ABO incompatible, respectively. Autologous PBSCs 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 is obtained by automated controlled-rate freezing, using dimethyl sulfoxide (DMSO) as the cryopreservative. PBSCs typically are stored in the vapor phase of liquid nitrogen; they are frozen in aliquots of 50–75 mL and 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 the thawing and the 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 1 mL/kg at one sitting (which translates to 10 mL/kg of PBSCs for components that were cryopreserved with 10% DMSO).

Key points

- As is the case with transfusion of any other blood product, the most important issue with regard to the safe infusion of HSCs is definitive bedside identification of the patient.
- To obtain optimal cell viability, frozen aliquots of HSCs must be thawed rapidly and infused into the patient without delay.

Clinical case

A 56-year-old woman is being evaluated for matched HSCT from her brother, for high-risk acute myeloid leukemia in first remission. She is A positive, and he is O negative. She is enrolled on 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 without any evidence of bleeding.

Transfusion support after HSCT

The intensity of transfusion support varies widely for different conditioning regimens; typically, 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 7-8 g/dL, although there has been no randomized clinical trial conducted to determine the optimal RBC transfusion threshold. The landmark studies that support a platelet transfusion threshold of $10 \times 10^9/L$ were conducted in patients undergoing leukemia induction. A recent multicenter randomized trial comparing different platelet transfusion doses (see the section Platelet Transfusion Dose), however, included both autologous and allogeneic HSCT recipients, who were transfused prophylactically at the $10 \times 10^9/L$ threshold. 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 cytokine-induced endothelial damage and activation of vWF; portal hypertension with hypersplenism further increases platelet transfusion requirements. The availability of the original PBSC donor in related

matched HSCTs can be a useful option in platelet-refractory patients.

Interest has been renewed in granulocyte transfusions since the mid-1990s, when it became possible to collect adequate doses of granulocytes in donors who are willing to undergo stimulation with G-CSF with or without corticosteroids. 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, and its use is not warranted given the potential risks of subjecting healthy individuals to G-CSF and corticosteroids. 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. An RCT is under way to study the incremental benefits of granulocyte transfusions in HSCT-related neutropenic infections.

All cellular blood components—RBCs, platelets, and granulocytes—must be γ -irradiated before transfusion in HSCT recipients to prevent t-GVHD. Some institutions with high-volume oncology and HSCT caseloads have elected to irradiate all platelets and RBCs to avoid the disastrous consequence of omitting this step as γ -irradiated components are safe for patients who are not at risk of t-GVHD. γ -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.

Prevention of CMV infection is an important part of transfusion management in CMV-seronegative HSCT recipients. Leukoreduction filters achieve a 3- to 4-log reduction of contaminating leukocytes in blood products. A landmark randomized comparison of leukoreduced-filtered versus CMV-seronegative blood components in CMV-seronegative HSCT recipients (with seronegative donors) found no significant difference in the incidence of CMV infection; others have argued that the study was not powered to detect differences in CMV disease, which was 2.4% in the filtered group and 0% in the seronegative group. With the improvement in CMV surveillance followed by preemptive antiviral therapy, which has further reduced the incidence of CMV disease after HSCT, this question will never be answered definitively. A subsequent cohort study, reported from the same center, in

which CMV-seronegative components were used preferentially when available, found that the number of filtered RBC units from CMV-positive donors was the primary predictor of transfusion-transmitted CMV infection, which occurred in 3% of the CMV-seronegative HSCT recipients. Preemptive therapy with ganciclovir after detection of antigenemia prevented all but one case of CMV disease, which was treated successfully, before day 100. Most transplantation centers, in practice, will use filtered blood components for CMV prevention; prestorage leukoreduction at the blood supplier is preferred to bedside filtration.

ABO-incompatible HSCTs

Allogeneic HSCTs do not require ABO matching because ABO antigens are not expressed on pluripotent stem cells. Because the genes for HLA are encoded on chromosome 6 and the genes for ABO are on chromosome 9, two siblings can have an identical HLA type but different ABO types. A recent 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 grades 2-4 acute GVHD in the ABO-identical or ABO-mismatched groups. A single-institution study that focused exclusively on nonmyeloablative regimens, however, 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 (in addition to other such factors as donor sex, age, CMV status) that ultimately dictates donor choice.

A major ABO mismatch occurs when the recipient's plasma contains antibodies against the donor's RBCs. An example would be a group O recipient–group A donor pair. Early investigators used large-volume plasma exchange followed by infusion of incompatible RBCs to lower the iso-hemagglutinin titer before infusing the donor marrow; subsequent groups depleted RBCs from the donor marrow in lieu of recipient antibody depletion. Either of these approaches can effectively prevent immediate hemolytic transfusion reactions during marrow infusion. ABO-mismatched HSCT recipients have a slightly slower neutrophil recovery. Mature erythroid progenitors do express ABO antigens, and immune-mediated delayed erythropoiesis

with reticulocytopenia can occur, leading to prolonged transfusion dependence up to 1 year after transplantation. The incidence is approximately 10%, and there is an inverse correlation between ABO hemagglutinin titers and reticulocyte counts, although attempts to lower hemagglutinin titers by plasma exchange have not been effective in treating RBC aplasia in this setting; extracorporeal immunoabsorption columns, cyclosporine withdrawal, erythropoietin, and rituximab also have been used.

A minor ABO mismatch occurs when the donor's plasma contains antibodies against the patient's RBCs (group A recipient–group O donor, such as in the illustrative case). Plasma depletion of the HSC product is performed by some centers, but acute hemolysis from donor plasma is uncommon. A more important concern is the phenomenon of immune hemolysis from mature, competent passenger lymphocytes transfused with the HSC component, especially with T-cell-depleted marrows, PBSCs versus marrow, the use of cyclosporine alone (without methotrexate) for GVHD prophylaxis, and reduced-intensity conditioning regimens. The hemolysis can occur abruptly, from days 7-14 after HSCT, and can be severe or even fatal. A positive DAT distinguishes passenger lymphocyte hemolysis from thrombotic microangiopathy after transplantation; some centers perform periodic DAT screening in minor ABO-mismatched HSCT recipients, although the effectiveness of this strategy 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 RBC antibodies such as anti-D have undergone transplantation with antigen-positive grafts using the same principle of RBC depletion if marrow is the HSC component. 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 sickle cell disease undergoing HSCT may present a challenge if they have developed multiple RBC alloantibodies or antibody to a high-incidence antigen. The optimal time to discontinue antigen-negative blood is not known, but one strategy is to wait until lymphocyte type is 100% donor because residual recipient lymphocytes may resume production of RBC alloantibodies with antidonor specificity.

It is important to consider alloimmune hemolysis in the differential diagnosis of hyperbilirubinemia in the post-transplantation patient because veno-occlusive disease and liver GVHD are likely to be more common occurrences. 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

Table 12-5 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
B	A	O	AB
A	O	O	A or AB
B	O	O	B or AB
A	AB	A or O	AB
B	AB	B or O	AB
AB	O	O	AB
AB	A	A or O	AB
AB	B	B or O	AB
Rh neg	Rh pos	Rh neg	Rh pos or Rh neg
Rh pos	Rh neg	Rh neg	Rh pos or Rh neg

HSCT = hematopoietic stem cell transplantation; neg = negative; pos = positive; RBC = red blood cell.

blood grouping during these situations. Table 12-5 provides useful guidelines for the selection of the appropriate blood group type for RBCs, platelets, and plasma for various scenarios of donor-recipient ABO-HSCT incompatibility.

Key points

- All cellular blood products administered to recipients of HSCT must be γ -irradiated to minimize the risk of potentially fatal t-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, pure red cell aplasia, or other adverse effects.

Pediatric transfusion issues

Hemolytic disease of the fetus and newborn

HDFN (or erythroblastosis fetalis) most commonly is due to maternal-fetal mismatches involving Rh or ABO antigens, which can cause an antigen-negative mother to mount an IgG antibody response against the antigen-positive fetal RBCs. These antibodies can be transported across the placenta and cause passively acquired immune-mediated hemolytic anemia in the fetus, leading to profound anemia and hydrops fetalis. Fetal demise may be seen in severe cases. The incidence of this disorder has been reduced dramatically with the use of antenatal and peripartum administration of anti-D to Rh(D)-negative mothers, which abrogates the maternal immune response to primary exposure to the D antigen. As a result,

most cases of HDFN now are attributed to Rh antigens other than D, as well as K1 (Kell blood group system), and ABO. ABO HDFN is characterized by hyperbilirubinemia with mild anemia (if any); the mother is typically group O with IgG anti-A,B alloantibodies (an antibody with cross-reactivity to both A and B antigens), and the infant is typically group A. ABO HDFN responds well to phototherapy. Cord blood should be ABO and Rh typed and a DAT should be performed for all infants born to group O mothers, all infants born to Rh-negative mothers (mother of an Rh-negative infant does not require anti-D prophylaxis), and all infants born to mothers with known (non-D) alloimmunization.

Intrauterine transfusion

Intrauterine transfusion (IUT) is much less commonly needed in the 21st century, and technical expertise should be concentrated in centers that specialize in high-risk obstetrics and perform IUT regularly. In a sensitized pregnancy, Doppler ultrasound and amniotic fluid studies guide the need for fetal blood sampling, which is performed after 20 weeks of gestation. Blood is prepared for IUT if the fetal hematocrit is <25%-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 cross-match. A fresh unit (≤ 5 days old) is used, either citrate-phosphate-dextrose-adenine (CPD-A) unit (without additive solution) or an additive solution unit with the supernatant removed. The unit must be γ -irradiated to prevent t-GVHD because the fetal immune system is immature; leukoreduction or a CMV-seronegative donor is used to provide a CMV-safe component. The unit should be negative for sickle hemoglobin. Once IUT is initiated, it is repeated every 3-4 weeks until 35 weeks to maintain fetal hematocrit at approximately 25%. Neonates who have undergone IUT will type as O negative; such neonates may have suppressed erythropoiesis, which necessitates 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 have made exchange transfusion for hemolytic disease of the newborn or for hyperbilirubinemia an uncommon occurrence. Appropriate unit selection follows the same principles for IUT described previously (ie, fresh O-negative unit, negative for any offending antigen, cross-matched against maternal serum, leukoreduced, irradiated, hemoglobin S negative). In addition, the RBCs are concentrated and reconstituted with group AB FFP, 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 much less efficient in lowering plasma bilirubin. Complications of exchange transfusion include hypocalcemia, dilutional thrombocytopenia, and catheter-related thrombosis, infection, or bleeding.

Alloimmune cytopenias in the fetus or neonatal period

Analogous to HDFN, maternal–fetal mismatches involving platelet-specific or neutrophil-specific alloantigen systems may result in NAIT or NAIN, respectively. The target antigens are quite diverse but often consist of membrane glycoproteins that are specific to the cell implicated in the immune cytopenia. The most common antibody specificity in NAIT in whites is anti-HPA-1a (PL^{A1}), which resides on the platelet fibrinogen receptor GPIIb/IIIa, although numerous other polymorphisms and specificities on this and other membrane constituents are documented in the literature. NAIN often is due to fetal–maternal mismatches involving the neutrophil-specific NA-1/NA-2 system. No prophylactic therapies currently are available for NAIT or NAIN.

Management of these disorders often includes antenatal maternal IVIg to reduce antibody levels, decrease placental transfer of antibodies, and reduce cellular destruction in the fetus. Transfusion support of NAIT is initiated with random donor platelets (which produce an adequate platelet increment in the majority of cases however short lived) in the setting of life-threatening bleeding (such as intraventricular hemorrhage, which can occur in utero). IVIg is a therapeutic option if the bleeding is mild to moderate. If subsequent platelet transfusion is needed, most often washed irradiated maternal platelets, prepared through apheresis, are obtained. Maternal platelets are essentially always negative for the target antigen in question; their use abrogates the need to wait for the often-lengthy serologic determinations that are required to identify platelet alloantibody specificity. Some blood centers have registries of specific platelet antigen-negative donors available for emergency apheresis if the mother is unable to donate.

Maternal ITP or autoimmune neutropenia can cause passively acquired immune thrombocytopenia or immune neutropenia in the fetus, respectively. It is important to rule out the latter disorders by checking a complete blood count, bone marrow biopsy, or appropriate antibody assays in the mother before using the mother as a source for platelets or neutrophils. The currently available assays for antiplatelet antibodies and antineutrophil antibodies are not always highly sensitive or specific and the diagnosis or exclusion of NAIN or ITP thus should not be based solely on the results of antibody assays.

Red blood cell transfusion in preterm neonates

The physiologic anemia of infancy occurs at 10–12 weeks, and the nadir hemoglobin is rarely <9 g/dL. Among preterm infants, this decline occurs at an earlier age, and the nadir is 7–8 g/dL; the physiologic nadir is compounded by phlebotomy blood loss. The blood loss through cumulative phlebotomy in a preterm infant's first weeks of life commonly exceeds the entire blood volume. Delaying umbilical cord clamping for 30–60 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. Limiting phlebotomy blood loss is a crucial part of minimizing transfusion in a preterm infant. Pretransfusion specimens for cross-matching are not necessary as long as a cord blood sample is available to determine the infant's ABO/Rh type and DAT. Erythropoietin has limited efficacy at best and appears to increase the risk of retinopathy of prematurity. There should be a program to limit donor exposure in neonatal transfusions. Typically, a fresh additive-solution O-negative unit (≤ 7 days old) is dedicated to one or two preterm infants and is used exclusively for all transfusions for those one or two infants for up to 42 days of allowable storage. Two randomized clinical trials of restrictive versus liberal transfusion criteria used transfusion thresholds that varied with patients' respiratory and other status and postnatal age. 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. Significantly, 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 one of the two trials demonstrated that a restrictive transfusion threshold increased the percentage of infants who avoided transfusion altogether (from 5%–11%). A subgroup analysis of infants in the restrictive transfusion group in the smaller trial had a higher incidence of apnea, severe brain injury, and mortality. Long-term follow-up of these infants would be important.

The fact remains that 90% of preterm neonates require a transfusion. Most U.S. centers routinely irradiate all cellular components for neonates for a variable period of time after birth (typically 4–6 months). Other centers base irradiation criteria on gestational age and birth weight. In addition, leukoreduced 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. Although 2,3-DPG is depleted in stored RBCs, it is rapidly regenerated after transfusion; infants given stored RBCs had stable 2,3-DPG levels after small-volume RBC transfusions.

Component therapy in neonates

Newborns may require FFP, most commonly for disseminated intravascular coagulation secondary to sepsis; 10–15 mL/kg produces a 15%–20% increase in factor level, assuming ideal recovery. If cryoprecipitate is required for persistent hypofibrinogenemia despite FFP, a dose of 1 unit should produce a 100 mg/dL increase in fibrinogen (in older infants, the cryoprecipitate dose is 1 unit per 5–10 kg of body weight).

Neonatal thrombocytopenia is not uncommon in preterm neonates, occurring in 22% of infants in one series. It is frequently a sign of sepsis or severe inflammation and often precedes other signs of sepsis. Prophylactic transfusions often are recommended in neonates with platelet counts <20,000–30,000/uL if otherwise stable; in unstable neonates or those requiring invasive procedures, platelets are transfused to maintain a count of ≥50,000/uL. The usual platelet dose in neonates is 10–15 mL/kg, which is one platelet concentrate (allowing for tubing volume). 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 should be washed to remove incompatible plasma. Routine volume reduction of platelets is not necessary or recommended because the procedure can jeopardize platelet quality.

Extracorporeal membrane oxygenation and congenital heart surgery

Extracorporeal membrane oxygenation is used to treat respiratory and cardiac failure in neonates secondary to diverse conditions, such as persistent pulmonary hypertension, congenital diaphragmatic hernia, and meconium aspiration syndrome. The membrane oxygenator consumes platelets, and RBCs are needed to prime the circuit and maintain a hematocrit typically around 40%. A program to limit donor exposure that includes using dedicated RBC units and aliquots of single-donor apheresis platelets can significantly decrease donor exposure, although the duration of extracorporeal membrane oxygenation drives the transfusion demands. Similarly, bypass cardiac surgery frequently is performed on small infants with congenital heart disease. Although smaller infants are more likely to require transfusion, transfusion-free procedures have been safely performed for infants as

small as 5 kg using miniaturized bypass systems and a variety of blood-conservation techniques.

Key points

- The immune system in the fetus and in neonates up to the age of 4 months is immature and probably is 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 utilizing modern technology to retrieve 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 versus liberal transfusion thresholds (7 g/dL vs. 9.5 g/dL) in pediatric ICUs found that a restrictive transfusion strategy was noninferior 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). Cardiac, craniofacial, and scoliosis surgery account for many cases of perioperative transfusion in older children; the older child or adolescent undergoing elective scoliosis surgery benefits from judicious use of autologous blood donation and intraoperative cell salvage in an integrated blood-conservation approach. Large-volume transfusion in the perioperative setting dictates careful monitoring of electrolytes and coagulation status. Hematologic or oncologic disease accounts for the remaining cases of pediatric transfusion. Pediatric patients with sickle cell disease and thalassemia benefit from leukoreduced blood transfusion to reduce HLA alloimmunization (and febrile nonhemolytic transfusion reactions); some evidence suggests that development of HLA antibodies is associated with the development of RBC antibodies in patients with sickle cell disease. They also benefit from the use of extended RBC phenotypic matching (for C, c, E, e antigens within the Rh system and K1) to reduce alloimmunization. Delayed hemolytic transfusion reactions from RBC alloantibodies can precipitate vaso-occlusive crises, hyperhemolysis, or autoantibody development.

Autoimmune hemolytic anemia

Clinical case

An elderly woman presents with an Hb of 6 g/dL and a positive DAT that reveals IgG, but not complement, on the surface of her red cells. Her reticulocyte count is <1%. She has never been transfused and has never been pregnant. She is started on prednisone for the treatment of presumed warm-type (IgG-mediated) AIHA. Because of shortness of breath and a previous history of heart disease, red cell transfusions are ordered. Multiple red cell cross-matches are incompatible. Three units of cross-match-incompatible leukoreduced red cells are transfused. The peripheral blood hemoglobin concentration increases to 9 g/dL, and she experiences no untoward reactions.

Transfusion in patients with AIHA can be challenging to the hematologist and the transfusion service. Autoantibodies to RBCs can result in multiple incompatible RBC cross-matches, which may lead blood banks to advise 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. In instances in which the patient previously has not been transfused or pregnant, however, alloantibodies to non-ABO antigens are unlikely to be present and patients usually can be transfused safely with ABO-compatible blood. Even in patients who previously have been transfused or pregnant, withholding transfusions because the cross-matches are incompatible may preclude the administration of lifesaving transfusions. Techniques are available to minimize the risk of transfusion in such situations; these techniques are important because failure to identify underlying alloantibodies in patients with AIHA not only leads to the destruction of the transfused donor cells but also can cause serious exacerbation of the concomitant autohemolytic process. Some transfusion services routinely perform extended RBC phenotyping—that is, the patient's RBCs are typed with regard to antigen systems in addition to ABO and Rh(D), at the time that a diagnosis of AIHA is first rendered, to facilitate the identification of alloantibodies that may appear subsequently. DNA-based methods are preferable when available.

Several studies have examined the incidence of clinically significant alloantibodies in multiply transfused patients with AIHA and found that up to 30%–35% of such patients develop alloantibodies. Thus, if a patient has received a transfusion or been pregnant, the transfusion service must perform specific testing to determine whether alloantibodies are present concurrently with the panagglutinating autoantibodies that typically are associated with AIHA. The term *panagglutinating* refers to the fact that most autoantibodies that cause AIHA will agglutinate most units of RBCs because

the antigenic target is typically a “public” antigen, that is, an antigen present on the RBCs of a large fraction of the population as a whole. The public antigen is often a common epitope on the Rh protein that is distinct from the common alloantigenic Rh epitopes. The most definitive technique for detecting alloantibodies in the presence of autoantibodies is called *autoadsorption*. With the autoadsorption technique, an aliquot of the patient's serum is adsorbed repeatedly with the patient's own erythrocytes and then tested for alloreactivity with panel or donor erythrocytes in a standard antibody screen.

If the patient has undergone transfusion recently, however, autoadsorption can be used only if sufficient quantities of recipient-derived reticulocytes can be harvested by a special density gradient centrifugation procedure that is not widely available. Otherwise, autoadsorption cannot be reliably interpreted because the transfused RBCs present in the prospective recipient's blood could adsorb the very same alloantibodies that the laboratory is attempting to detect. In this situation, a method called *differential adsorption* is used. Differential adsorption involves adsorbing different aliquots of patient serum against RBCs of different 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. Because most warm-reacting autoantibodies react with erythrocyte-surface determinants that do not vary among patients (ie, public antigens), adsorption with erythrocytes 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 along with an autoantibody, both the autoantibody and the anti-Jk^a antibody will be adsorbed by Jk^a-positive adsorbing cells, but only the autoantibody will be 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 alloantigens, even though the patient is positive for the alloantigen in question. This preferential reactivity can be striking, such that if the autoantibody has relative specificity for a particular antigen, the antibody may demonstrate much stronger reactions against antigen-positive RBCs in comparison with antigen-negative RBCs. The preferential reactivity may be so strong that autoantibody reactivity against RBCs negative for the antigen in question cannot be detected.

The apparent specificity demonstrated by autoantibodies is often to an antigen in the Rh blood group system, most commonly to the e antigen. These antibodies are referred to as *mimicking* antibodies. In these situations, 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, blood lacking the antigen in question generally is not available. In this situation, blood transfused through a blood warmer usually will survive 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 workup 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). 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, note that the patient's reticulocyte count was relatively low. A substantial minority of patients will manifest at least transient reticulocytopenia early in the course of AIHA, a phenomenon that may be due to the fact that autoantibody titers may increase more quickly than the bone marrow can generate a reticulocyte response or due to rapid destruction of reticulocytes by the autoantibody.

Key points

- Red cell transfusions in patients with life-threatening AIHA should not be withheld simply because all available units are cross-match incompatible.
- 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 immune thrombocytopenia (commonly known as ITP) is problematic because the efficacy of platelet transfusions in such patients is unpredictable because of the potential destruction of transfused platelets by the autoantibody. (As is the case with AIHA, the autoantibody in ITP often reacts with public antigens.) Transfusion of platelets in patients with ITP usually is attempted only in patients with life-threatening hemorrhage. Administration of IVIg may improve the survival of transfused platelets in patients with ITP, and the administration of IVIg or continuous infusions of platelets have been tried in patients with life-threatening hemorrhage and patients

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. Recent introduction of the thrombopoietin mimetics (eltrombopag and romiplostim) has further expanded the therapeutic armamentarium for ITP.

Except in life-threatening bleeding situations, such as intracranial hemorrhage, platelet transfusions should be avoided in consumptive thrombocytopenias, such as TTP and heparin-induced thrombocytopenia, because they could exacerbate the thrombotic process that characterizes both of these disorders.

Sickle cell disease

Patients receiving long-term transfusion therapy often become alloimmunized to multiple blood components, including RBC antigens, leukocyte antigens, and plasma proteins. For reasons that are not entirely clear, this is especially true in patients with sickle cell disease, of whom 2%-50% become alloimmunized to non-ABO RBC antigens. Leuko-reduced blood products should be used to minimize febrile reactions. Patients with sickle cell disease, however, are particularly prone to alloimmunization against RBC alloantigens and 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 unusual phenotypes. The reasons for the high rate of alloimmunization to RBC antigens among patients with sickle cell disease are poorly understood. Likely factors include repeated exposure to RBC antigens in combination with differences in the frequencies of certain RBC antigens between the predominantly white donor population and the predominantly black patient population. Patients with sickle cell disease also may have a higher intrinsic responsiveness to blood group antigens.

Indications for transfusion in sickle cell disease include stroke, acute chest syndrome, aplastic crisis, and preoperative preparation to reduce the risk of postoperative respiratory complications. Sickle cell 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 carries the risk of additional donor exposures and requires adequate vascular access.

Techniques for preventing and managing alloimmunization in patients with sickle cell disease are controversial. In many institutions, RBCs from patients with sickle cell disease are subjected to extended antigen typing for the most important antigen systems in addition to ABO and Rh(D), including Kell, Kidd, Duffy, MNSs, Rh(C), Rh(E), and others, before transfusion therapy is initiated. DNA-based methods

may be preferable when available, particularly in multiply transfused patients. Extended RBC phenotyping facilitates future identification of antibody specificities and the transfusion of at least partially extended antigen-matched units, which may reduce the incidence of subsequent alloimmunization. Most commonly, matching is performed for the extended Rh antigens (C, c, E, and e) and the major Kell antigen (K), in part because providing RBC units that are matched for these antigens does not require use of phenotypically rare blood units. It usually is not possible to match routinely for all antigens for which the patient has been typed because most blood centers that identify and store phenotypically rare blood provide these units only to patients with demonstrated alloantibodies because of the limited supplies of rare-phenotype blood. The adoption of high-throughput blood group genotyping platforms by more blood centers will facilitate extended blood group matching between blood donors and sickle cell patients, particularly coupled with minority donation recruitment efforts.

The development of alloantibodies is occasionally associated with autoantibody formation, which complicates transfusion therapy. The prevalence was 8% in a pediatric series of sickle cell patients; about half the patients with detectable autoantibody had evidence of hemolysis, often associated with a positive DAT for complement. Hyperhemolysis is another transfusion-related complication observed in sickle cell disease, often presenting with severe anemia and reticulocytopenia 7–10 days after an index transfusion. The hematocrit is typically lower than the pretransfusion value, indicating the destruction of autologous RBCs. 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. Many centers that treat patients with sickle cell disease have observed an apparent association between DHTRs, the onset of sickle cell crises, and the occurrence of other complications of sickle cell disease.

Key points

- Patients with sickle cell disease on a chronic transfusion program should receive extended-match red cells to prevent alloimmunization to non-ABO red cell antigens.
- DHTRs caused by alloimmunization to non-ABO red cell antigens represent a significant problem in chronically transfused patients with sickle cell disease.
- Simple transfusion to a hemoglobin of 10 g/dL is indicated in patients with sickle cell disease who have early acute chest syndrome or who are undergoing prolonged surgical procedures. Exchange transfusion is indicated in patients with major complications, such as severe acute chest syndrome or cerebral infarction.

Massive transfusion

Massive transfusion is defined as the replacement of one blood volume within 24 hours, typically 4–6 hours after severe trauma or major surgery. Coagulopathy of massive transfusion is multifactorial: hypothermia, acidosis, the dilutional effect of blood loss and inadequate coagulation factor replacement, reduced hepatic synthesis of coagulation factors in massive hepatic injury, disseminated intravascular coagulopathy from hypotension and tissue injury, and consumption of coagulation factors or platelets. Unfortunately, both laboratory tests and transfusion volume do not correlate well with the severity of bleeding. In the absence of hypovolemic shock and significant liver dysfunction, the exchange of one circulating plasma volume does not reduce the clotting factor activities below levels necessary to maintain hemostasis, that is, approximately 50% of the normal levels. Thrombocytopenia is the most frequent abnormality associated with massive transfusion. When transfusions of 1.5–2.0 blood volumes are administered over 4–8 hours, the mean reduction in the peripheral blood platelet count is approximately 50%. Ideally, it is preferable to obtain appropriate coagulation tests, including prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), and plasma fibrinogen level, to guide plasma 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. Some institutional massive transfusion protocols also incorporate the off-label use of recombinant factor VIIa, typically at a lower dose than that used in hemophilia patients with inhibitors, although there is no consensus on this use and thromboembolism is a potential adverse effect. 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. Platelet abnormalities account for most cases of correctable nonsurgical bleeding following such procedures. Platelet dysfunction and aggregation may result from platelet contact with the foreign 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. Dilution by priming the extracorporeal circuit with nonblood solutions may reduce the platelet count by as much as 50%. 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. As a result of these issues, platelet transfusion to correct quantitative or qualitative platelet defects is the mainstay of treatment of nonsurgical bleeding associated with cardiopulmonary bypass procedures. Even if routine blood testing reveals significant coagulation abnormalities, such testing will not detect qualitative defects in platelet function. In addition, because platelet products contain significant quantities of plasma, platelet transfusion alone still may be the treatment of choice even when the primary laboratory abnormalities appear to be coagulation factor related. Routine transfusion of platelets to patients who are not bleeding and are not severely thrombocytopenic does not appear to be justified. Thromboelastography offers whole blood-based coagulation testing that can pinpoint the coagulation defect to a deficiency in platelets, coagulation factors or fibrinogen, or excessive fibrinolysis. In selected centers, its use has reduced indiscriminate transfusion of plasma or platelets in nonsurgical bleeding in perioperative or postoperative cardiac surgery patients.

Transfusion risks

Clinical case

Shortly after the initiation of a red cell 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 red cell product and the patient reveals that the product is type A, the patient is type O, and the cross-match is incompatible.

Acute hemolytic 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 urine, and laboratory evidence of intravascular hemolysis. As noted, ABO isoantibodies are complement fixing and lead to

the intravascular destruction of the transfused RBCs, as manifested in the previous patient by hemoglobinuria and hemoglobinemia. 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 DAT that demonstrates both IgG and complement on the surface of the recipient's RBCs. Disseminated intravascular coagulation also occurs and bleeding may result. Patient misidentification due to clerical error 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.

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 before a subsequent RBC transfusion. Following the subsequent transfusion, the patient develops an anamnestic immune response to the mismatched antigen, leading to delayed destruction of the transfused RBCs. Clinical symptoms of hemolysis including fever, anemia, and jaundice develop 7–10 days after the transfusion; the link to the preceding transfusion is not always obvious. 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 disseminated intravascular coagulation have been reported. The antibodies most often implicated in DHTR are directed against antigens in the Rh (34%), Kidd (30%), Duffy (14%), Kell (13%), and MNSS (4%) antigen systems.

Febrile reactions

Multiparous women and multiply transfused patients commonly develop leukoreactive antibodies that cause nonhemolytic febrile reactions to RBC or platelet transfusions. In addition, during the storage of blood, clinically significant quantities of cytokines (IL-2, IL-6, IL-8, and tumor necrosis

factor) are sometimes liberated from donor-derived passenger leukocytes present in platelet and RBC products. Prestorage leukoreduction, as opposed to poststorage bedside leukofiltration, may reduce the accumulation of these bio-mediators and the probability of febrile, hypotensive, or hypoxic transfusion reactions. Febrile transfusion reactions usually are self-limited, and in most cases, the clinician can administer subsequent transfusions without undue risk. The main concern is that an elevation in temperature during a transfusion, although most likely the result of this innocuous febrile transfusion reaction, cannot be distinguished from an evolving life-threatening acute hemolytic transfusion reaction in which fever can be the only clue. 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.

Allergic reactions

Minor allergic reactions manifested by urticaria are frequent in multiply transfused patients. Antihistamines generally alleviate symptoms of allergic reactions, but they have not been shown to prevent them. Many urticarial reactions are donor specific and thus do not recur with subsequent transfusions. If a recipient experiences multiple urticarial reactions, the clinician should consider premedication with antihistamines or washed products resuspended in albumin and saline in severe cases. The clinician, however, must take consider the possibility that washing platelets may impair platelet function.

Severely IgA-deficient patients may make anti-IgA antibodies that can cause anaphylactic reactions. This is a rare occurrence, however, considering the number of patients at risk. When such a patient is encountered, extensively washed RBCs must be given, and plasma products from IgA-deficient donors must be transfused. Platelets can be collected from IgA-deficient donors to avoid platelet-washing procedures. 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 antigranulocyte antibodies (in particular, anti-HLA antibodies), cytokines, biologically active lipids, or other substances. The resulting clinical picture is *noncardiogenic pulmonary edema*. Signs and symptoms include dyspnea, hypoxemia, hypotension, fever, and a chest x-ray showing bilateral infiltrates with

pulmonary edema. Aggressive pulmonary support, including intubation and mechanical ventilation, frequently is needed. Approximately 80% of patients improve within 48–96 hours, 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 patient-derived nor donor-derived antibodies can be identified, and other mechanisms have been advanced such as the priming of neutrophils by bioactive lipids that accumulate during blood storage.

In 2006, TRALI represented approximately 50% of all transfusion-related fatalities reported to the U.S. FDA. Although the true incidence rate of TRALI is unknown, it may occur in as many as 1 in 5,000 transfusions of any plasma-containing blood product (ie, packed RBCs, platelet concentrates, and FFP) with a 5%–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 brought on by the transfusion. A consensus definition of TRALI is acute lung injury (ALI) occurring during a transfusion or within 6 hours of completion with no other temporally associated causes of ALI. ALI is defined as a syndrome of: (i) acute onset, (ii) hypoxemia ($\text{PaO}_2/\text{FiO}_2 < 300$ mm Hg, O₂ saturation less than 90% on room air, or other clinical evidence), (iii) bilateral pulmonary infiltrates, and (iv) 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 those first cases of TRALI by those donors, however, would require the elimination of all blood donors whose plasmas contain anti-HLA or neutrophil antibodies. Because screening donors for such antibodies would be extraordinarily expensive and time consuming, an approach has been investigated in which FFP simply is not prepared from female donors whose plasmas have a greater chance of containing HLA antibodies due to pregnancy. When this approach was adopted in the United Kingdom in late 2003 where 60% of TRALI cases previously had been caused by FFP transfusions, no reports of TRALI deaths due to plasma occurred after 2004 (six deaths occurred in 2005, none from plasma). Recent studies investigating the relationship of HLA antibodies in blood donors to pregnancy and transfusion history have shown that the incidence of class I and class II HLA antibodies is about the same in nontransfused males, transfused males, and females who have never been pregnant (0.9%, 1.2%, and 1.5%, respectively). The incidence of HLA antibodies dramatically increased with an increasing number of pregnancies (11.2%, 23.1%, and 28.1%

with 1, 2, and ≥ 3 pregnancies, respectively). Major blood suppliers in the United States now limit the use of female plasma for the production of FFP to decrease the incidence of TRALI, or screen for HLA and 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 will not be eliminated. Therefore, strict transfusion criteria for plasma, early recognition, and prompt clinical management are key to dealing with this potentially fatal transfusion reaction.

Transfusion-associated circulatory overload

Dyspnea 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), which is underdiagnosed or at least under-reported, to hospital blood banks as a transfusion reaction. Risk factors for TACO include extremes of age, history of cardiac disease, and transfusion of multiple blood components within a relatively short period of time. An elevated brain natriuretic peptide (BNP) may be helpful. Therapy consists of diuretics and decreased blood administration rate utilizing split units, in which the blood bank prepares half a unit of blood to be given over the same amount of time as a full-size unit.

Infectious complications

Bacterial and protozoal 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 became recognized as the most common cause of transfusion-associated morbidity and mortality due to an infectious source 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 became 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 have involved 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. Efforts to detect the presence of bacteria in platelet units before dispensing to a

patient include culturing an aliquot of the unit and using a rapid strip immunoassay for bacterial antigens. Other less sensitive methods for detection using a surrogate marker for evidence of bacterial metabolism, such as a low pH, in an aliquot of the platelet suspension have been discontinued.

In recent years, several 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 in the transfusion recipient.

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 1-3 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 and widely implemented, often in conjunction with the donor questionnaire. Transfusion-transmitted babesiosis has been reported in New England and has been identified in patients receiving platelets, refrigerated RBCs, and even frozen-thawed RBCs. A number of investigational tests are being evaluated for donor screening in areas endemic for *Babesia*. *Borrelia burgdorferi*, the etiologic agent of Lyme disease, has yet to be confirmed as having been transmitted by blood transfusions.

Hepatitis

Despite the elimination of commercial blood donors and screening of donor blood for hepatitis B and hepatitis C, posttransfusion hepatitis occasionally still develops. Although transmitted in a similar fashion to hepatitis B, acute transfusion-related hepatitis C infection is subclinical and anicteric in most cases. Hepatitis C infection frequently becomes chronic, however, and often results in clinically significant liver dysfunction. Recent data suggest that patients who have undergone HSCT are at much increased risk for late-onset cirrhosis after hepatitis C exposure.

With current anti-hepatitis C virus antibody tests and nucleic acid testing, it is estimated that the risk of posttransfusion hepatitis C is 1 per 1.8 million units transfused. The risk of hepatitis B transmission by transfusion using current test methods is 1 per 220,000. Table 12-6 summarizes the estimated risks of various transfusion-associated infections.

Table 12-6 Infectious complications of transfusion.

Infectious agent	Approximate risk per transfused unit
Hepatitis B	1:220,000
Hepatitis C	1:1.8 million
HIV-1, HIV-2	1:2.3 million
HTLV-1, HTLV-2	1:2,993 million
Bacterial sepsis	1:75,000 (platelet transfusions); 1:250,000 to 1:10 million (red blood cell transfusions)
<i>Babesia</i>	1:1,800 in endemic areas
Malaria (<i>Plasmodium</i> spp.)	1:4 million

HIV = human immunodeficiency virus; HTLV = human T-cell lymphotropic virus.

A number of recent studies have examined the utility of photochemical treatment of platelets with amotosalen and ultraviolet A to inactivate HIV, hepatitis viruses, and other viral pathogens. To date, this approach appears both efficacious and relatively sparing in terms of qualitative platelet function, although decreases in quantitative platelet recovery have been observed in some studies. Concern with the use of these viral inactivation methods focuses more on the potential untoward systemic effects of residual pathogen-inactivation agents introduced during transfusion.

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 2.3 million. Nucleic acid amplification testing for HIV has reduced the window of serologic conversion from 16 days to 8 days. Because the HIV-1 RNA response detected by nucleic amplification testing occurs before or simultaneously with p24 antigen and because HIV-1 RNA is detected after p24 disappearance, p24 testing has been replaced by this newer technology. The availability of heat-treated concentrates, solvent detergent-treated products, and recombinant factor concentrates has eliminated AIDS as a risk for hemophiliacs.

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 was initiated in 1989. 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 3 million.

West Nile virus

During the 2002 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 have implemented 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

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) is not eliminated by solvent detergent treatment.

Cytomegalovirus

Passenger leukocytes that inevitably contaminate RBC and platelet products, albeit in relatively small numbers, 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, HSC transplantation recipients, and other highly immunosuppressed patients. The risk of acquiring CMV from transfusions is particularly high when pretransplantation serologic testing reveals that neither the HSC 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 HSC 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 HSC transplantations. Other institutions simply use leukoreduced blood products in all such recipients, regardless of CMV status. The latter strategy has the additional advantage of reducing the risk of alloimmunization to HLA antigens and thus of developing refractoriness to platelet transfusions. None of these strategies to reduce the risk of CMV transmission, however, eliminates the necessity of γ -irradiating blood products administered to the same subsets of patients to prevent the development of t-GVHD.

Transfusion-associated graft-versus-host disease

t-GVHD is an important risk in patients undergoing treatment of hematologic malignancies, patients undergoing HSCT, and patients with certain congenital immunodeficiency syndromes. The pathophysiology of t-GVHD is thought to involve engraftment of small numbers of donor-derived passenger leukocytes in a host whose immune system is unable to eliminate the passenger leukocytes. Unlike *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 t-GVHD develops, mortality approaches 100% as a result of the severe pancytopenia that usually develops. Patients may develop signs and symptoms of classic transplantation-associated GVHD, including skin rash, diarrhea, liver function test abnormalities, and other symptoms. The infusion of any cellular blood product can theoretically cause t-GVHD. γ -Irradiation of all cellular blood products before transfusion—but not conventional leukoreduction—virtually eliminates the risk of t-GVHD.

t-GVHD also has been described in immunocompetent patients when the donor is homozygous for an HLA haplotype shared with the recipient. Transfusion within relatively inbred populations, such as in Japan, appears to increase the risk of t-GVHD because of the increased prevalence of donors who are homozygous for an HLA haplotype shared with the recipient. This appears to set 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 leukocytes recognize the nonshared HLA allele on the recipient's cells and thereby 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 t-GVHD. Therefore, directed donations of cellular blood products from blood relatives should be γ -irradiated.

Blood management

Clinical case

A 44-year-old multiparous female requires orthopedic surgery. Pretransfusion compatibility testing reveals antibodies to three separate red cell antigens, K1 (Kell system), Fy^a (Duffy system), and E (Rh system). Cross-match-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

Clinical case (continued)

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 ever-broadening 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 induce 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 also may be indicated in some cases. Avoidance of iatrogenic anemia by limiting 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 1-2 units of RBCs per week. Critical thought should be applied to ensure that all blood tests ordered are justified and actively contribute to patient care. The frequency, timing, and volumes of blood draws, including use of lower volume blood collection tubes when appropriate, also should be coordinated to limit the volume of patient blood collected for testing.

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 autolo-

gous 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 “just because they could not hurt.” Use of preoperative autologous blood donation is now broadly discouraged as approximately 50% of autologous units never are transfused, and patients who donate autologous units preoperatively may present to surgery with anemia that increases the 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 be any safer than regular banked units in terms of transmitting infectious agents. In addition, blood from first-degree relatives must be irradiated to prevent t-GVHD. For these and other reasons, directed donations are also now 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 red cell salvage or perioperative autotransfusion. ANH involves removal of one 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 red cell 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 anticoagulated reservoir and then washed with normal saline. The washed salvaged RBCs are concentrated for reinfusion to the patient. When using cell salvage techniques, precautions need to 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 Witnesses will consent to autologous transfusion using a continuous cell salvage circuit, potentially allowing for more complex surgeries to be performed in this group. The technique can be helpful in patients for whom it is difficult to find compatible blood because of the presence of multiple red cell antibodies.

Judicious transfusion

If all measures to avoid allogeneic transfusion have been exhausted and transfusion is clinically indicated, care should be taken to transfuse the least amount of blood products required to achieve the desired outcome. Clinicians must keep in mind that 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 of 50% without difficulty. Guidelines and expected outcomes for transfusion are discussed elsewhere, but one of the major behavioral changes incorporated into most blood management programs is a shift in practice from transfusing RBC units in multiples of two to 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, or their equivalents, 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.

Erythropoietic-stimulating agents can be used in Jehovah’s Witness 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, erythropoietic-stimulating agents, and possibly the newer thrombopoietic growth factors, 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.
- Transfusing only when absolutely indicated (right product to the right patient at the right time and for the right reason) can help avoid unnecessary risks associated with transfusion.
- Preoperative medical management of anemia before elective surgery can reduce perioperative transfusions.
- Preoperative autologous donation generally is discouraged due to wastage of collected units, residual risks of clerical error, bacterial contamination, and volume overload and due to the preoperative anemia associated with these donations.

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Cellular basis of hematopoiesis and stem cell transplantation
Dan S. Kaufman and David T. Scadden

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CHAPTER
13



Cellular basis of hematopoiesis and stem cell transplantation

Dan S. Kaufman and David T. Scadden

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Introduction and historical perspective

Humans produce approximately 300 billion blood cells per day. The source of these blood cells is hematopoietic stem cells (HSCs) that are estimated to number in the tens of thousands and reside in the bone marrow (BM). Although the field of stem cell biology has grown dramatically over the past decade, hematologists have been using HSCs clinically for >50 years in the form of bone marrow transplantation (BMT). Identification of HSCs in the BM emerged after the dropping of the atomic bombs in 1945 when it was recognized that many people who survived the explosions died of hematopoietic failure resulting from radiation damage. Subsequent studies in rodents starting in the 1950s demonstrated that transplanting spleen or BM cells into irradiated animals prevented radiation-induced hematopoietic failure. 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 of myeloid, erythroid, and megakaryocytic cells in the spleens of the recipients 7–14 days after transplantation (Figure 13-1). These colonies were shown to arise from a single implanted cell, were capable of extensive proliferation, and could be retransplanted into secondary recipients. Although later work showed that these cells (called spleen colony-forming unit [CFU-S] cells) likely represent short-term, rather than long-term, hematopoietic repopulating

cells, these studies laid the foundation for concepts of hematopoietic (and nonhematopoietic) stem cell biology.

Hematopoietic stem cell concepts

Hematopoietic stem cell properties

Stem cells are defined by two key properties: the ability to self-renew and to differentiate. 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. Studies in mice demonstrate that HSCs can be serially transplanted for many generations between recipients. To provide key functions, however, HSCs must differentiate into all of the mature cells of the hematopoietic system. These mature lineages include erythroid cells for oxygen transport, myeloid and lymphoid cells that provide immune defense, and for the megakaryocytes and platelets essential for hemostasis.

Stem cell assays

As the field of stem cell biology has grown over the past decade, it is useful to recognize how studies of HSCs and hematopoiesis (blood development) have pioneered many key concepts in this field. A spectrum of complementary *in vitro* and *in vivo* assays has been developed to identify and characterize hematopoietic stem and progenitor cells.

Colony-forming assays

The identification of a cell capable of clonal differentiation *in vivo* by Till and McCulloch (1961) prompted other groups

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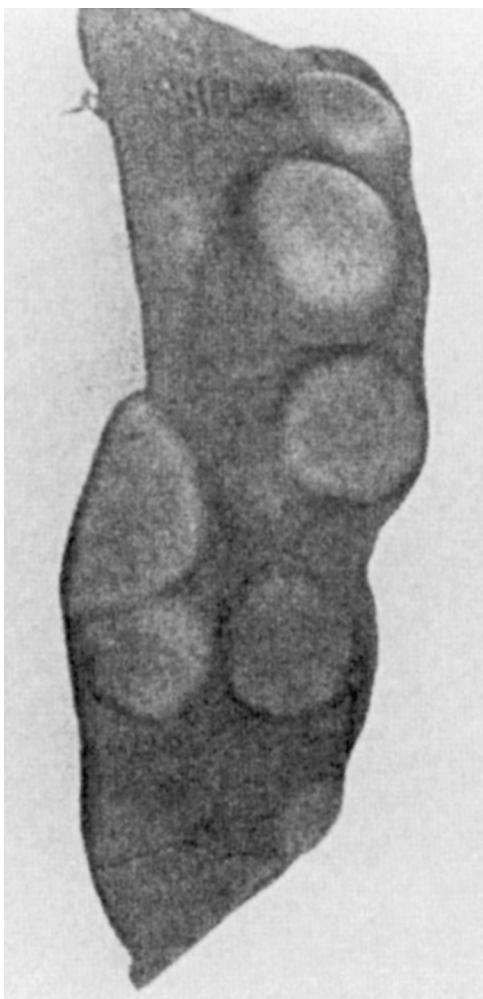


Figure 13-1 Spleen colony-forming unit (CFU-S) assay. Macroscopic splenic hematopoietic colonies arising from the CFU-S stem/progenitor cell 14 days after injection of murine BM into lethally irradiated mice. Reproduced with permission from Williams DA. Stem cell model of hematopoiesis. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Cohen HJ, eds. *Hematology: Basic Principles and Practice*. New York, NY: Churchill Livingstone, 1995.

to develop a simple quantitative assay for the growth and differentiation of single-cell suspensions of mouse BM in vitro (Figure 13-1). When hematopoietic cells were cultured in a semisolid medium (typically soft agar or methylcellulose based), discrete colonies were formed and included cells in multiple stages of differentiation (Figure 13-2). 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 CFU or colony-forming cell (CFC). In contrast to the self-renewal potential of most CFU-S, colonies grown in vitro showed more limited ability to proliferate in secondary cultures. This limitation implied that the most primitive stem cells failed to survive or proliferate in this assay. Therefore, CFCs were suggested to define

a population of committed progenitors, fed from an earlier, more immature compartment of HSCs.

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 will be 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 similarity 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. As this work originally involved transplanting human hematopoietic cells into severe combined immune-deficient (SCID) mice, the engrafting cells were termed SCID-repopulating cells (SRCs). Although newer, more immunodeficient mouse strains typically now are used for these studies, the term SRC is still used. SRCs are considered more primitive and clearly distinct from prior multipotent primitive human hematopoietic cell populations identified using in vitro methodology. The mouse strain NOD-SCID/IL-2R γ ^{-/-} (commonly termed NSG or NOG mice) is now used for these analyses. Recent studies have identified the phenotype of a single human hematopoietic cell that can reconstitute all

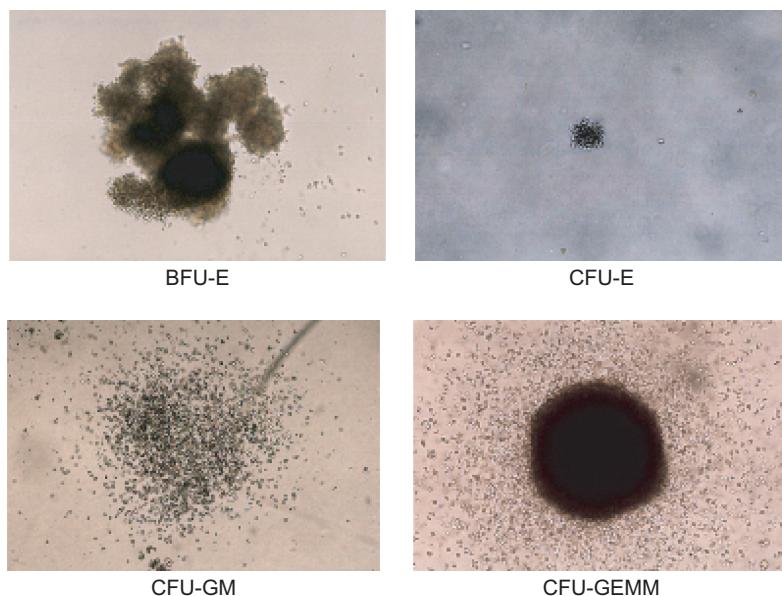


Figure 13-2 Examples of colony-forming assays of human hematopoietic progenitors cells. These include CFU erythroid (CFU-E), burst-forming unit erythroid (BFU-E), CFU granulocyte/macrophage (CFU-GM), and CFU granulocyte/erythroid/ macrophage/ megakaryocyte (CFU-GEMM). Reprinted with permission from Stem Cell Technologies, Inc.

hematopoietic lineages when transplanted into NSG mice. These CD34⁺CD38⁻Lin⁻CD45RA⁻Thy1⁺Rho^{lo}CD49f⁺ cells represent as close to a human HSC that currently can be identified phenotypically. (Here, Lin⁻ refers to cells that lack expression of mature hematopoietic lineage antigens and Rho^{lo} designates cells that efflux the mitochondrial dye rhodamine-123.) 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 hematopoietic development.

HSC dynamics

The most primitive HSCs are rare, representing approximately 1 in 10⁴-10⁶ BM cells. During normal steady-state hematopoiesis, adult HSCs cycle slowly and are relatively resistant to cytokine stimulation. When they are recruited into active hematopoiesis, they exit the G0 phase of the cell cycle, and their daughter cells may either be a replicate of the parent cells (self-renewal) or enter into a differentiation program. This distinctive, asymmetric division process is the basis for long-term preservation of stem cells 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 more limited in the types of cells they produce, and they are lineage restricted. They also are progressively limited in a self-renewal capacity

but have rapid proliferative ability and cytokine responsiveness. These features make progenitors the population that most readily responds to stress conditions to up- and down-modulate production of specific blood cell types.

Different types of progenitor cells are responsible for production of specific blood cell types. In the setting of advanced age, animal models indicate that the relative abundance of specific types of progenitors may change. Lymphocyte-producing progenitors may diminish relative to myeloid-producing progenitors, potentially contributing to immune alterations with age.

Hematopoietic niche

Hematopoietic cell development from HSCs 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 a number of cell types. 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. For example, mature osteoblasts are important in stem cell mobilization, nestin-positive mesenchymal stem cells are important for HSC persistence, leptin receptor-positive mesenchymal cells are important for c-kit ligand production (also called stem cell factor) and adipocytes have been implicated as negative regulators of HSC number.

Other cell types, such as neural cells of the sympathetic nervous system and nonmyelinating Schwann cells, also play

a role in HSC support or localization. The sympathetic nervous system mediates circadian modulation in the number of HSC moving from BM to blood stream on a daily basis. Mature hematopoietic cells are thought to influence HSC functions in the BM. Specifically, macrophages are participants in HSC mobilization into the blood and T-cells are thought to influence HSC engraftment and relative protection from immune attack. Therefore, a complex admixture of cells participates in what is designated 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 c-kit ligand 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 stem cells, 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 either harvesting stem cells by mobilization into the blood or delivery of transplanted stem cells to enable engraftment.

HSC circulation, homing, and mobilization

Stem cells migrate from one site of blood cell production, circulate, home, and enter other supportive sites. This certainly is the case in development before the existence of bones in the fetus. The fetal liver serves as the site of hematopoiesis in cases in which HSC niches must exist. Cells eventually transit from this site to nascent BM to establish hematopoiesis, and they are thought to transit via the bloodstream. Even after

BM is the site of hematopoiesis, HSC traffic into and out of the BM regularly. Experiments using parabiotic mice, in which the circulations of two separate mice are joined surgically, have indicated that murine HSCs exit the BM and transit the peripheral blood (PB) system at surprisingly high flux rates (estimated to be $\sim 10^4\text{-}10^5$ long-term repopulating HSCs [LT-HSCs] per day in a mouse). Other mouse studies have identified macrophages and osteoblasts as cells critical for G-CSF-mediated effects on HSC trafficking through regulation of the SDF1-CXCR4 axis.

This ability of HSCs to move from the BM to the PB stream is exploited for collection of stem cells for clinical hematopoietic cell transplantation. Instead of needing to collect BM as a source of HSCs, “mobilized” PB collected by apheresis procedure provides a rich source of HSCs. Different preparative regimens and growth factors (eg, granulocyte colony-stimulating factor [G-CSF]) can be used before this collection to increase the number of HSCs, with enumeration of CD34⁺ cells used as a surrogate marker of HSCs. Plerixafor, a drug that blocks the chemokine receptor CXCR4 from binding to stromal derived factor-1 (SDF-1), is now used clinically when needed to increase the numbers of circulating HSCs (given with G-CSF). The rate, timing, and destination of the HSC that circulate from the BM to periphery appears to involve chemokines and their receptors, especially SDF-1 and its receptor CXCR4; integrins, particularly Very Late Antigen-4 (VLA-4, also termed integrin $\alpha 4\beta 1$); selectins, such as P-selectin glycoprotein ligand-1 (PSGL1, also termed CD162) and the calcium-sensing receptor and the intracellular-signaling molecules, G α s and Rac1/Rac2. A number of agents have now been shown to alter stem cell mobilization and at least one, the prostaglandin E2, is being tested to enhance engraftment. This is one means by which

Bone marrow hematopoietic niche components to date

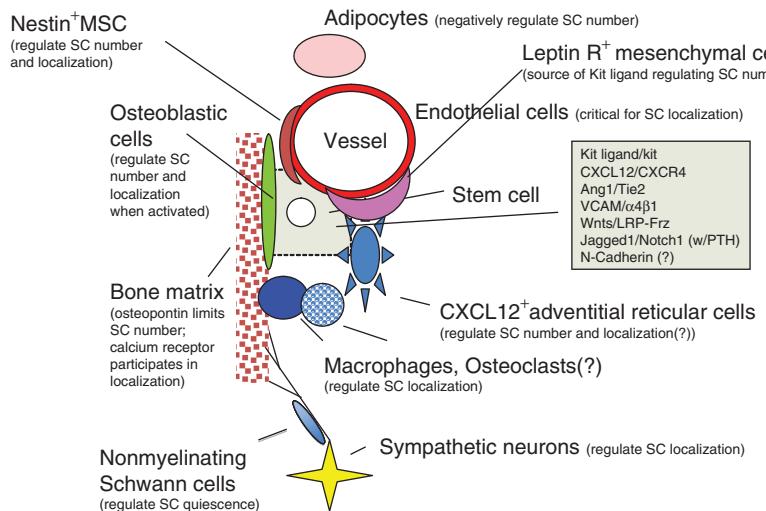


Figure 13-3 Hematopoietic stem cells (HSC) localize to perivascular spaces some of which are near the endosteal surface. A number of mouse models have been used to define specific cell types and gene products that, when manipulated, result in a change in HSC location or number. The cell types are indicated in the figure with the HSC function 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.

studies of stem cell–niche interactions ultimately may affect clinical medicine, reducing the numbers of stem cells needed for transplantation through more efficient engraftment.

Hierarchical differentiation of HSCs

A complex network of transcription factor and growth factor signaling pathways regulates HSC self-renewal, lineage commitment, and differentiation. Identification of numerous surface antigens on different hematopoietic cell populations combined with use of flow cytometry or other cell separation methods have been used to prospectively isolate cell populations with selective potentials. These studies, in combination with different *in vitro* and *in vivo* hematopoietic assays, have led to a now-traditional hierarchical map of

hematopoiesis shown in Figure 13-4. Although it initially was thought that these cell populations were committed irreversibly to their downstream lineages, more recent studies show these developmental stages are not strictly binary branch points. Some progenitor cell populations may have more flexible developmental potential, depending on the assay and readouts used.

Summary

Hematopoiesis involves a regulated set of developmental stages from HSCs that produce hematopoietic progenitor cells that then differentiate into more mature hematopoietic lineages, which provide all the key functions of the hematopoietic system. Hematopoietic reconstitution during

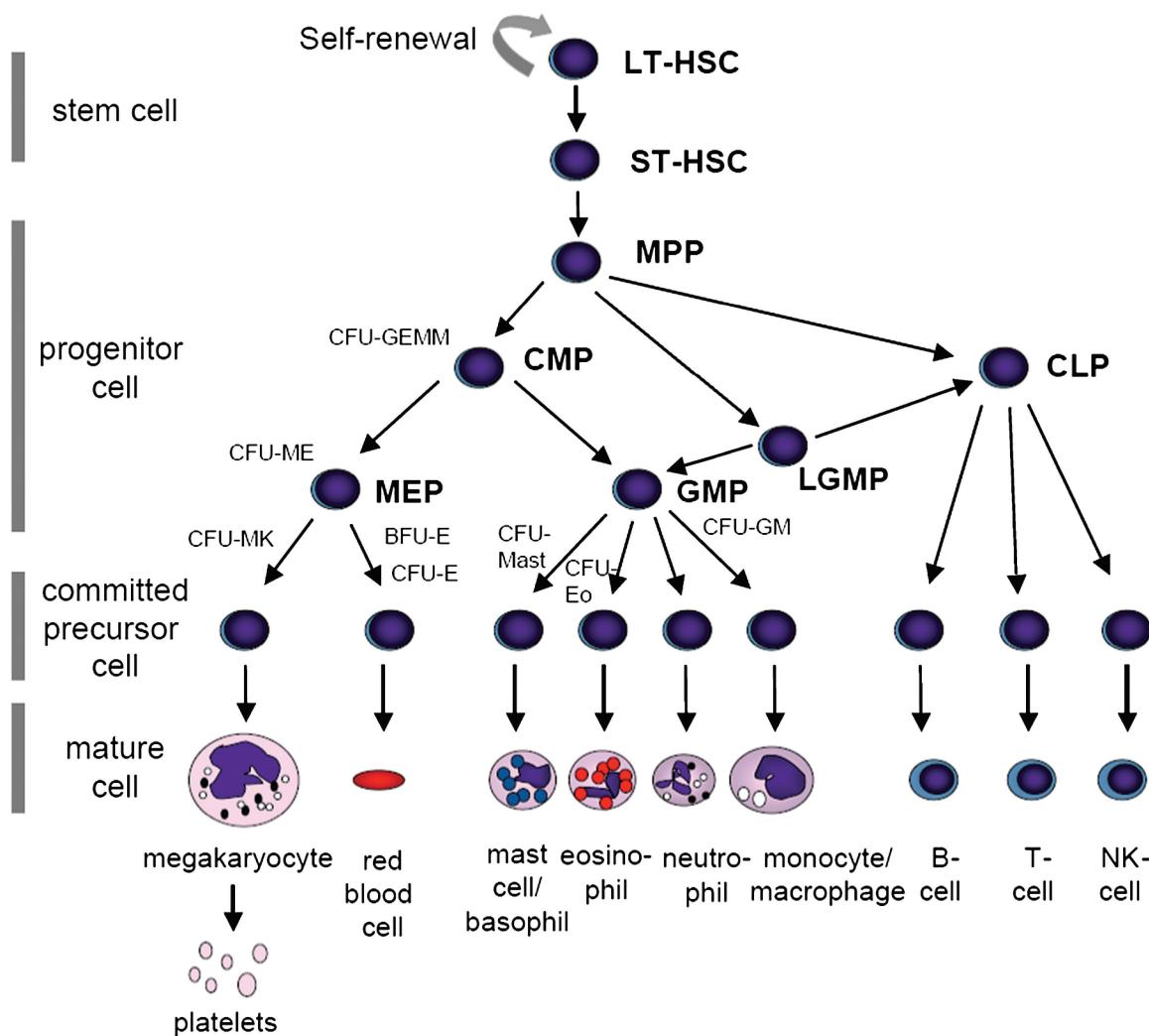


Figure 13-4 Classical hierachical map of hematopoietic development. Schematic diagram depicting hierachical relationships between immunophenotypically and functionally defined hematopoietic cell populations. Corresponding CFCs identified by *in vitro* assays are indicated. Surface antigen phenotypes of human hematopoietic stem and progenitor cells also indicated. CLP = common lymphoid progenitor; CMP = common myeloid progenitor; GMP = granulocyte-monocyte progenitor; LT-HSC = long-term repopulating hematopoietic stem cell; MEP = megakaryocyte-erythroid progenitor; MPP = multipotential progenitor cell; NK = natural killer; ST-HSC = short-term repopulating hematopoietic stem cell.

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 stable repopulation being provided by long-lived, multipotent HSCs. Long-term hematopoiesis is sustained by a relatively small number of HSCs.

Key points

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 even after transplantation.
- Multipotency: the ability to make all types of blood cells.
- Relative quiescence: the ability to serve as a deep reserve of cells to replenish short-lived, rapidly proliferation progenitors.
- *In vivo* transplantation models are currently the only reliable assays of HSC activity.

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.
- Lineage commitment and thereby, limited cell-type production.

Key features of the HSC niche:

- Anatomically and functionally defined regulatory environment for stem cells.
- Modulates self-renewal, differentiation, and proliferative activity of stem cells, thereby regulating stem cell number.
- Niche function is important in maintaining hematopoietic integrity and 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.

Ontogeny of hematopoiesis

The primary sites of hematopoiesis change in a temporally and spatially ordered fashion during development in most vertebrates. In humans, the yolk sac serves as the initial site of erythropoiesis from weeks 3-6 of gestation. The primary site of hematopoiesis then shifts to the fetal liver from 6-22 weeks, and finally to the BM, which becomes the predominant and lifelong site of blood cell production. Erythroid development during the yolk sac phase has been termed *primitive erythropoiesis*, whereas development during fetal liver and adult BM stages is referred to as *definitive* or *adult erythropoiesis*. Primitive erythrocytes differ from definitive erythrocytes in a number of ways, but most notably in the

expression of specific embryonic globin genes. Additional studies suggest that primitive hematopoietic development gives rise to some myeloid and lymphoid cells (eg, natural killer cells). Interestingly, the developmental potential of hematopoietic cells produced in the yolk sac closely resembles hematopoietic cells derived from human embryonic stem cells (hESCs) and induced pluripotent cells (iPSCs). Additional research on this topic is needed, however.

Sequential development of hematopoietic sites led to the belief that the complete prenatal and postnatal blood system originally were produced from yolk sac-derived stem cells. Subsequent experimental data, however, indicate that definitive hematopoiesis arises from an independent intraembryonic source of stem cells. These HSCs develop during a brief developmental window at approximately 4-5 weeks of gestation in the vicinity of the developing aorta termed the *aorto-gonadal-mesonephros* (AGM) region. Studies in mice and zebrafish indicate that these HSCs develop directly from specialized “hemogenic” endothelial cells lining the ventral aspect of the dorsal aorta. Indeed, recent imaging advances demonstrate these hematopoietic cells directly budding from the endothelial cells of the AGM. It is believed that these HSCs then seed the developing fetal liver, where they expand and differentiate into committed progenitor cells. It is not clear whether AGM-derived HSCs also directly seed the developing BM or whether they first must 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.

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 other features. These differences may have implications regarding choice of stem sources for human transplantation therapies.

Key points

- 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 age of the organism.

Stem cell characteristics

Attempts to purify stem cell populations have used a combination of approaches based on the physical and biologic properties of HSCs.

Physical properties

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, commonly are used as a preenrichment step in stem cell purification protocols.

Biologic properties

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 flurouracil markedly reduces progenitor cells, while relatively sparing populations enriched in HSC activity.

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. 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.

Immunophenotype

Combinations of cell surface markers have been used to enrich for HSC populations. 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 LT-HSCs (Flk2⁻) from short-term repopulating HSCs (ST-HSCs; Flk2⁺). Other protocols have used the SLAM family receptors CD150, CD244, and CD48 to isolate murine HSCs that are highly purified as CD150⁺CD244⁻CD48⁻. This SLAM phenotype does not translate for isolation of human HSCs.

Early studies to isolate human HSCs found that approximately 1% of human BM cells express CD34. Isolation of CD34⁺ cells enriches for CFC in vitro and hematopoietic engraftment when transplanted into irradiated nonhuman primates. Similarly, human CD34⁺ selected cells contain stem cells capable of fully reconstituting the lympho-hematopoietic system in humans after myeloablative chemotherapy and

radiation therapy. Approximately 5%-25% of CD34⁺ cells also express low to moderate levels of Thy-1 antigen. As in the mouse, Thy-1 expression by human hematopoietic cells decreases with differentiation, and most lineage-restricted progenitors are CD34⁺Thy-1^{+/low} cells. Additional studies demonstrate that human HSCs do not express mature cell lineage markers (Lin⁻) or CD45RA or CD38. Isolation of CD34⁺CD38⁻Lin⁻ cells provides a relatively easy method to sort for putative HSCs based on in vitro and in vivo studies. This remains, however, a heterogeneous population. Sorting for the integrin CD49f further enriches for HSCs. Isolation of CD34⁺CD38⁻Lin⁻CD45RA⁻Thy1⁺Rho^{lo}CD49f⁺ cells provides a potentially pure population of HSCs in which a single cell can fully engraft NSG mice. These detailed cell-sorting methods are useful to identify genes and signaling pathways that mediate human HSC development. Clinical sorting to such high purity is not likely to be necessary, however, as use of CD34⁺ cell-based isolation allows relatively efficient magnetic enrichment of HSCs. Some clinical studies have done more specific isolation of CD34⁺Thy1⁺ cells to try to avoid collection of contaminating tumor cells. Although these studies were encouraging, this requires a flow cytometry-based isolation procedure that is difficult to do on a widespread basis.

Heterogeneity of HSCs

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 have 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 studies. Studies of HSCs during development and aging demonstrate changes in HSCs developmental potential, gene expression and growth factor requirements in the early postnatal period, as well as in aged individuals. Additionally, HSCs demonstrate epigenetic changes over time, likely leading to functional differences, such as a relative lymphoid deficiency in the elderly. Epigenetic changes may be part of the development of hematopoietic diseases, such as myelodysplastic syndrome. Indeed, the ability of hypomethylating agents such as azacitidine and decitabine that can be used to treat MDS confirms an epigenetic basis for this disease. Advances in cell sorting, genetic analysis, and other technologies are making analysis of HSCs increasingly precise. This progress certainly will provide additional insight into HSC biology and heterogeneity.

Ex vivo expansion of HSCs

Because of the limited number of HSCs available from some sources, there has been great interest over the years to define conditions allowing ex vivo expansion of HSCs. Such systems require the ability to increase the number of HSCs without sacrificing any of the properties of HSCs, such as self-renewal, proliferative capacity, or ability to differentiate into all mature blood cell populations. Previous studies demonstrated that combinations of hematopoietic cytokines (such as stem cell factor, Flt-ligand, thrombopoietin, or G-CSF), with or without stromal support cells, could provide modest expansion of putative HSCs in culture. Clinical trials using these strategies typically demonstrated some modest increase in neutrophil recovery, but did so without long-term engraftment of expanded HSCs. Other endothelial expressed proteins, such as angiopoietin-like 5 and insulin-like growth factor binding protein 2, have been shown to expand bona fide HSCs cultured from mice. These agents, however, have not yet been translated into clinical studies.

Recently, considerable progress on this front has been made, with at least three different novel strategies for ex vivo expansion of HSCs going into clinical studies. One agent is an antagonist of the aryl hydrocarbon receptor (AHR). AHR is an intracellular receptor that binds dioxin and similar xenobiotics. Previous studies had demonstrated that AHR can regulate expression of many hematopoietic genes and signal pathways. Treatment of UCB CD34⁺ cells with the AHR antagonist (termed StemRegenin1) lead to a 17-fold increase in SRC potential. Notably, this AHR antagonist could expand putative HSCs from humans and nonhuman primates, but not mice. This illustrates important species-specific differences in hematopoietic pathways. Clinical trials to expand UCB with this agent are ongoing. The Notch pathway has been actively studied to expand HSCs. Translational studies of this pathway have focused on use of the immobilized Notch ligand Delta1. Culture of UCB CD34⁺ cells with immobilized Delta1 and cytokines lead to a 16-fold increase in SRCs. Clinical studies using a double-UCB transplant with one unit expanded by culture with Delta1 and the other unit unmanipulated lead to more rapid engraftment as measured by time to absolute neutrophil count (ANC) $\geq 500/\mu\text{L}$. Although it might be expected that the initial rapid engraftment would be due to myeloid cells from the expanded unit, in some cases, that unmanipulated unit provided initial neutrophil recovery. Most patients in the phase 1 clinical trial demonstrated long-term engraftment from the unmanipulated UCB unit, although some showed persistence of the Delta1 expanded cells. These trials suggest that these ex vivo expansion strategies may be valuable to hasten recovery of neutrophils (and possibly red blood cells and platelets). HSCs may be lost, however, in the expanded cell population.

A third expansion agent in recent clinical trials is prostaglandin E2 (PGE2). Interestingly, PGE2 was identified in a zebrafish screen for agents that increased HSCs. PGE2 (or more biologically stable derivatives of PGE2) subsequently was shown to increase hematopoietic development from mouse and human embryonic stem cells. Treatment of mouse BM, human UCB, and nonhuman primate mobilized PBSCs all demonstrate an increase in CD34⁺ cell and expansion of HSCs using in vivo transplantation assays. Clinical trials using the double-UCB transplant system are ongoing. Much of the increase in HSC engraftment due to PGE2 treatment may be from increase expression of adhesion molecules on the HSCs leading to improved trafficking to the hematopoietic niche. Future clinical translation may benefit from a combination of these and other approaches.

Number of stem cells required for engraftment

Normal hematopoiesis is characterized by many simultaneously active stem cells, each contributing a small proportion of the blood cells present at any given time. In contrast, the hematopoietic system regenerated in animals that have undergone transplantation is, after an initial period of clonal disequilibrium, characterized by the contribution of a relatively few stem cells. Monoclonal hematopoiesis also has been documented in humans after allogeneic hematopoietic cell transplantation (HSCR). Experiments in which mice were injected with varying numbers of unseparated marrow cells or purified stem cells, however, have shown that the number of clones contributing to hematopoiesis increases in proportion to the number of stem cells injected. Limiting numbers of repopulating stem cells in a graft may force such cells to exhibit a larger proliferative response than stem cells in a transplantation that exceeds minimal requirements. Although it may be possible to regenerate a functioning immunohematopoietic system after transplantation of relatively few stem cells, it is less likely that these cells are capable of sustaining hematopoiesis for the life span of the patient without periodic replacement. Therefore, although in clinical transplantation the adage appears to be “the more stem cells, the better,” the practical questions are, “How many stem cells are enough?” and “How are such cells measured?” In practice, mobilization of stem cells can be monitored by the total number of nucleated cells, CFU-granulocyte macrophages (CFU-GMs), or CD34⁺ cells to optimize harvesting time and achieve maximum yield. Successful engraftment of BM or mobilized PB cells in the clinical setting is predicted by a minimal dose of 2.5×10^5 CFU-GMs or 2×10^6 CD34⁺ cells/kg of recipient body weight. For UCB transplantation, having a cell dose $> 1.7 \times 10^5$ CD34⁺ cells/kg is beneficial to ensure engraftment in a reasonable time frame. With all

these cell sources, increased cell dose can correlate to time of engraftment and fewer transplant-related complications.

HSC transplantation (HSCT) is an accepted curative treatment modality for patients with selected malignant and nonmalignant diseases. Allogeneic and autologous HSCT have been used with great success in the management of diseases that otherwise were incurable. With the advent of improved supportive care, reduced-intensity conditioning regimens, and alternative sources of stem cells, the role of HSCT continues to evolve and to become more available to more patients in need.

Stem cell sources

BM harvest was the original method for harvesting HSCs for clinical transplantation, but mobilized PB stem cells and umbilical cord blood cells now are used commonly. The term *hematopoietic stem cell transplantation* (HSCT) or *hematopoietic cell transplantation* (HCT), instead of the original term *bone marrow transplantation* (BMT), now are used more often to encompass these various sources of stem cells. To preserve the acronym, BMT now can stand for *blood and marrow transplantation*. The basic goal of each of these methods is to replace host marrow cells (creating complete chimerism) or to supplement them (resulting in mixed chimerism). Donors can be related or unrelated and can be matched or mismatched at the major histocompatibility complex (MHC).

Donor types

Related donors

For patients needing an allogeneic HCT, a complete HLA-matched sibling donor is almost always the first choice. HLA-matched siblings usually lead to less GVHD and therefore to less morbidity and mortality. Some recent studies, however, have not found a significant survival advantage with matched siblings as compared with 8/8 or 10/10 HLA allele-matched unrelated donors. Matched sibling donors often are easier logically to coordinate for timing of transplantation. Decreased time to transplant may offer a survival advantage in some cases, such as for patients with leukemia who are in a tenuous remission. Using strict 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. For this reason, the chance that two siblings are HLA matched is slightly less than 25%. Parents usually are only haploidentical, but parental donors can be matched more closely with their offspring if by chance the parents share certain HLA alleles or even a complete haplotype.

Unrelated donors

For the majority of patients who lack a matched related donor, an HLA-identical unrelated donor often represents the next-best option. One of the major advances in unrelated HCT has been the establishment of donor marrow registries. Millions of potential donors have been HLA typed and are listed with national and international registries. The utility of these registries has been greatest for patients with common haplotypes where, because of linkage disequilibrium, the haplotype is found frequently within the registry. Thus, for patients with common HLA types, it now is 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.

Using donor registries often requires 3-4 months of search time until transplant because potential donors must be contacted and their willingness to serve as donors confirmed. Confirmatory typing must be performed, a mutually agreeable date for the transplantation must be selected, and the donor undergoes a history and physical examination. For many patients with aggressive disease, the time required to find the donor is prohibitive.

Outcomes of unrelated-donor transplantation are greatly influenced by the degree of donor-matching. The continued refinement of molecular typing has resulted in more precise identification of the best donor, resulting in lower rates of GVHD and graft failure in patients highly matched to their donors. There is now agreement that outcomes are optimal when a donor and recipient are matched completely at the allele level (ie, as determined by molecular typing) in HLA-A, -B, -C, -DR, -DQ (for a 10/10 molecular match). With each additional mismatch in A, B, C, or DR, the risks of transplantation increase, and few centers are willing to accept more than one antigen mismatch between an adult unrelated donor and recipient. The importance of matching for DQ and DP is less well established. Because of differences in minor histocompatibility antigens between donors and recipients, transplantation with unrelated donors still is associated with a higher incidence of GVHD and graft failure even when donor and recipient are matched perfectly.

Cord blood

Because of the inability to identify a fully matched related or unrelated donor in a timely fashion, additional sources of stem cells continue to be explored. Despite continued expansion of unrelated donor registries, it is estimated that only about one-third of patients in need of an unrelated donor will be able to find a match. Therefore, UCB cells harvested from the placenta and umbilical cord of newborns represent

a rapidly growing source of HSCs. UCB is a rich source of HSCs capable of hematopoietic reconstitution. Cryopreserved units of UCB typically can be obtained much faster than unrelated adult donor units can (available on average in 13.5 days in contrast to a matched unrelated donor search of 3–4 months). UCB contains relatively fewer T-cells responsible for GVHD, and the T-cells that are present are more immunologically naïve. Therefore, UCB demonstrates less allogeneic reactivity responsible for GVHD compared with marrow or PB grafts.

The initial UCB transplants were performed in young children using HLA-identical siblings and resulted in rapid engraftment and a low incidence of GVHD. Mismatched related cord transplants have a low incidence of GVHD as well, with two- and three-antigen mismatches well tolerated in children. Subsequently, unrelated donor cord blood was established as a source of stem cells with low rates of acute and chronic GVHD. As a result, both private and public cord banks have been established, allowing for the successful collection, typing, freezing, and dispensing of cord blood units. Because HLA mismatching between cord blood and recipients is better tolerated compared with marrow and PB grafts, the likelihood of finding a suitable donor for each patient is greater.

The greatest limitations of UCB transplantation are the slow rate of engraftment, prolonged myelosuppression, and delayed immune reconstitution, which results in higher rates of death from infection; all of these limitations are related to the relatively low hematopoietic stem or progenitor cell dose in cord blood. This low progenitor cell dose initially hampered the ability to obtain rapid engraftment in patients more than 50 kg.

In the past decade, use of UCB for adults has become more common. Multiple studies have now demonstrated that use of two cord blood units that provide a suitable cell dose leads to neutrophil engraftment within an average of 20–25 days. GVHD typically is less frequent when using UCB as a donor source. As UCB contains fewer T-cells than BM or PB, and the T-cells are more immunologically naïve, UCB only needs to be 4/6 HLA matched to the recipient. Additionally, because UCB is available in frozen UCB banks, the cells typically are available for transplantation more quickly than can be done if an unrelated adult donor is used. Clinical trials have demonstrated that overall survival of patients receiving UCB is similar to those who receive related or unrelated adult donor grafts.

Routine cord blood banking for newborns as a precaution for potential treatment of illness later in life commonly is advertised by private companies. Currently, the American Academy of Pediatrics recommends that private banking be used only in families where there is an affected child of a disease known to be curable by allogeneic transplantation.

Others should be encouraged to donate the cord blood to public banks.

Key points

Advantages of unrelated donor:

- Increased number of transplanted cells.
- More rapid time to engraftment.
- Improved immune reconstitution.
- Additional cells available for CD34 cell “boost” of donor lymphocyte infusion.

Advantages of cord blood:

- More readily available, shorter time to get to transplant.
- Less GVHD.
- Use of double-cord blood transplant decreases time engraftment.
- Double-UCB transplants provides a platform for UCB expansion.

Haploidentical-related donors

Haploidentical-related donors share one haplotype with the recipient, and their use is considered investigational. Parents are always haploidentical with their children, and siblings have a 50% chance of being haploidentical. It is estimated that approximately 90% of patients will have an available haploidentical donor.

The major challenges associated with haploidentical transplantation are the high rate of 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. Graft failure has been reduced with the use of large doses of stem cells and intensified conditioning regimens. Encouraging results have been reported, particularly in myeloid malignancies and in patients in remission at the time of HSCT.

Selection of bone marrow or mobilized peripheral blood

Use of mobilized PB as a source for transplantable HSCs predominates over BM as the major source of stem cells. PB results in more rapid engraftment (because of more committed progenitors being collected) and therefore less early complications. A recent randomized, phase 3 trial found that there was no survival difference at two years between patients who had received PB versus BM. There was evidence of more frequent chronic GVHD from PB and more graft failure from BM.

In a similar retrospective study of children of the International Bone Marrow Transplant Registry, poorer outcome was seen in all groups of patients receiving mobilized PB

grafts. Prospective studies to evaluate this issue in children are needed urgently. In addition, in the pediatric population, the donor is frequently a minor, for whom the risks of G-CSF and leukapheresis (the smaller the size of the patient, the more technically difficult the collection due to the fluid shifts involved) must be weighed against the risk of a marrow harvest. A recent report found no major untoward events for the healthy sibling donors evaluated following PBSC mobilization. The Pediatric Bone Marrow Transplant Consortium currently is investigating the ability to decrease the time to engraftment of marrow grafts through the use of G-CSF mobilization before donor harvest.

In adult and pediatric patients receiving transplantations for nonmalignant conditions (such as aplastic anemia) or where rapid engraftment is not a critical issue, BM may be a superior source of stem cells due to the lower rates of acute and chronic GVHD.

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 all hematopoietic cells). Studies with mESCs have been invaluable to identify genes that regulate hematopoietic development such has been done with mouse knockout studies. 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 genetically have not been manipulated. Overexpression of certain transcription factors, however, most notably HoxB4, have been shown to produce hematopoietic cells capable of engraftment in syngeneic recipients. Some additional factors 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 two switching events during embryonic-fetal development, whereas the mouse undergoes only one switching event. hESCs also have raised considerable interest because of the potential to use these cells to produce large amounts of human cells and tissues suitable

for transplantation or transfusion medicine. For example, there has been considerable interest in using hESCs to produce RBCs or platelets as an adjunct to the 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 effective engraftment of HSCs by transplantation into immunodeficient mice to any reasonable extent. Even genetic manipulation and over-expression of transcription factors, such as HoxB4, that is 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.

Induced pluripotent stem cells (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” that are able to convert the somatic cell population into cells that look and act essentially like embryonic stem cells. These studies were first done from mouse cells in 2006 and then from human cells in 2007. Like their ESC counterparts, iPSCs have been used to derive diverse hematopoietic cell lineages. Again, to date, transplantable HSCs have not been derived from iPSCs. This field will continue to mature in the future, 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 analyzed. This may lead to effective therapies based on using the iPSCs as a screening resource and would not require direct transplantation of iPSC derived cells. Additionally, future developments may allow for derivation of iPSCs from individuals with hematologic or others diseases and use these cells to produce essentially autologous replacement cell populations. This is an area that will develop more in the future.

Summary

The HSC niche is a critical aspect of the regulated production of blood cells throughout life. It is complex tissue in which multiple cell types and extracellular matrix proteins contribute to balance the molecular cues that govern stem cell 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. Ongoing efforts to improve stem cell engraftment into the niche and to discern how the niche contributes to disease are contexts in which research likely will contribute to future application.

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Clinical bone marrow and stem cell transplantation
Sergio A. Giralt and Jerald P. Radich

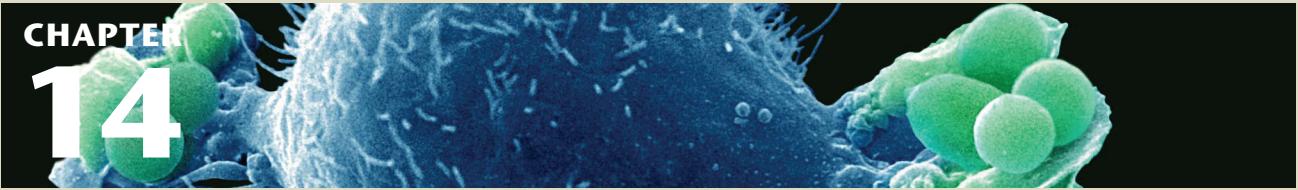
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CHAPTER
14



Clinical bone marrow and stem cell transplantation

Sergio A. Giralt and Jerald P. Radich

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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 pre-clinical research in hematopoiesis and hematopoietic stem cell transplantation (SCT) 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 irradiated hosts.

Notwithstanding these initial observations, the initial clinical experience with hematologic SCT was dismal with most patients succumbing from transplant-related complications. It was not until the discovery and identification of human leukocyte antigens (HLAs) as well as improvements in supportive care with the development of antibiotics and

antifungals that successful hematopoietic SCT in the form of bone marrow transplantation (BMT) could be a reality for sufficient patients to warrant its large-scale study. The landmark paper from Thomas et al. (1979), demonstrating that long-term disease control could be achieved in patients with refractory acute leukemias with the use of high-dose chemo-radiotherapy followed by infusion of HLA identical sibling marrow, marks the beginning of modern hematopoietic SCT.

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 five- to tenfold overcomes the resistance of tumor cells against lower doses. High-dose chemotherapy aims to destroy the tumor cells in an expediently timely manner to prevent the emergence of resistant clones. In 1978, investigators from the National Cancer Institute were the first to report the use of high-dose chemotherapy followed by autologous BMT for patients with relapsed lymphoma. These encouraging results were the initial clinical evidence that led to the widespread application of autologous SCT.

The hematopoietic stem cell

Hematopoietic stem cells (HSCs) are capable of reconstituting and maintaining a complete and functional hematopoietic system over extended periods of time. They are characterized by three intrinsic properties: extensive proliferative capacity,

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Off-label drug use: Dr. Giralt: Drug therapy for stem cell transplantation. Dr. Radich: not applicable.

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 HSC has been characterized by the following:

1. Ability to form multilineage colonies in semisolid soft agar medium.
2. Ability to form colony-forming units (CFUs) 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.

The HSCs account for 1 in 10,000 bone marrow cells, and during normal steady-state hematopoiesis, are in the G0 phase. Through a series of chemical signals they are recruited into active hematopoiesis and undergo a series of maturational cell divisions that culminate in the generation of progenitor cells with progressively limited self-renewal, proliferative, and 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 seven distinct families have been identified, including collagens, proteoglycans, fibronectin, tenascin, thrombospondin, laminin, and hemonectin. Within the marrow microenvironment, two types of *niches* have been described that favor HSC self-renewal versus 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 HSC 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 potentials.

Stem cells migrate from one site of blood cell production, circulate, 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 HSCs into the peripheral blood system and their collection by apheresis for HSC transplantation.

The stem cell transplant process

The SCT process is a complex procedure in which an individual receives a combination of chemical and physical agents to eliminate a malignant disorder or a poorly functioning bone marrow supported by reinfusion of HSC from the patient or a donor. Intense medical therapy is required as patients recover from the effects of the conditioning regimen and throughout the period of profound immune-suppression that occurs while the transplanted HSC mature and recover normal function. The components of the SCT process are listed below:

Stem cell transplant recipient

SCT is performed not only for patients with a variety of hematologic malignancies, but also for patients with non-malignant hematologic disorders. The most common indications for SCT are summarized in Table 14-1.

Having a condition that could be amenable to treatment with hematopoietic SCT is not enough to deem a patient *transplant eligible*. Transplant eligibility is determined by an extensive pretransplant evaluation of organ function and psychosocial evaluation to assess the risk-benefit ratio of poietic SCT compared with alternative treatment approaches. Table 14-2 summarizes the most commonly used criteria to determine SCT eligibility.

Types of stem cell transplants

Hematopoietic SCT traditionally has been classified according to the source of stem cells as either autologous or allogeneic.

Autologous stem cell transplantation

Autologous SCT involves using HSCs obtained from the same recipient. The stem cells can be obtained directly from the recipient's marrow or through mobilization of stem cells from the marrow into the peripheral blood using G-CSF and the CXCR4 antagonist plerixafor.

Table 14-1 Most common indications for stem cell transplantation.

Autologous stem cell transplantation		Allogeneic stem cell transplantation	
Diagnosis	No. of Procedures Performed in the United States in 2009	Diagnosis	No. of Procedures Performed in the United States in 2009
Myeloma	4,700	Acute myeloid leukemia	2,500
Non-Hodgkin lymphoma	2,800	Acute lymphoblastic Leukemia	1,100
Hodgkin lymphoma	1,250	Myelodysplastic syndrome	1,000

Table 14-2 Commonly used eligibility criteria for stem cell transplantation.

Eligibility criteria	Test	Transplant eligible	Comments
Patient performance status	History and physical exam	Usually ECOG 2 or less Karnofsky performance status of greater than 70%	Transplant related mortality is related to pre-SCT performance status. Patients with poor performance status generally are not considered candidates for SCT unless chances for long-term cure are high if SCT is successful.
Disease and disease status	Multiple	Depending on disease and disease status. Patients with high-risk disease and high-risk disease status have <10% chance of 2-year survival	Patients with advanced refractory disease are generally not considered transplant eligible. Armand et al. (2012) recently proposed a disease and disease status risk classification for stem cell transplantation.
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 may be changing with the advent of effective antiviral therapy (entecavir and highly active antiretroviral therapy). Prior hepatitis exposure does not affect transplant outcomes.
Cardiac function	Echocardiogram Nuclear medicine testing	Ejection fraction greater than 40%	Patients with cardiac history may require more extensive pretransplant evaluation, including referral to cardiology for stress testing or Holter monitoring.
Pulmonary function	Pulmonary function testing	No uncontrolled cardiac disease DLCO >40%	In some series, the most important predictor of outcome is DLCO <40%.
Renal function	Creatinine and creatinine clearance	Usually creatinine clearance >40 cc/min	Patients with poor renal function (including patients with ESRD) can be considered for SCT on a case-by-case basis. Autologous SCT is routinely performed in patients with multiple myeloma on dialysis.
Hepatic function	Liver function tests (transaminases and total bilirubin)	Bilirubin less than 2-3 × ULN unless history of Gilbert's disease	Elevated liver function tests predict liver toxicity.
Comorbidity scoring	Hematopoietic specific comorbidity index	No cutoff determined	Comorbidity scoring more useful to decide regimen intensity. HCT-CI most commonly used scoring system.
Psychosocial evaluation	Various	Varies with institutional guidelines	Essential to determine risk of noncompliance as well as caregiver availability and social support needed throughout the transplant process.

ECOG = Eastern Cooperative Oncology Group; SCT = stem cell transplantation; PCR = polymerase chain reaction; HIV = human immunodeficiency virus; HTLV-1 = human T lymphotropic virus; CMV = cytomegalovirus; EBV = Ebstein-Barr virus; DLCO = diffusion lung capacity; ESRD = end stage renal disease; ULN = upper limit of normal; HCT-CI = hematopoietic cellular therapy-comorbidity index.

Allogeneic stem cell transplantation

Allogeneic SCT involves using HPSCs obtained from a third party who can be a related or unrelated donor.

The choice between performing an autologous SCT versus an allogeneic source of SCTs is a difficult one. In general, diseases that affect the marrow or are difficult to cure with chemotherapy alone (ie, aplastic anemia, acute and chronic leukemia, genetic disorders) will require a third-party 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 to alkylating agents is observed and the role of a GVT effect is less certain (ie, lymphoma, myeloma, and germ cell tumor) the use of autologous stem cells is generally preferred.

Human leukocyte antigen typing

Determination of HLA types has become much more accurate 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 major histocompatibility complex (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.

The ideal donor is identified according to HLA compatibility as determined by HLA typing. The MHC refers to the

entire genetic region containing the genes encoding tissue 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 two 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 two polymorphic chains: an α -chain and a β -chain (both encoded on chromosome 6). Both chains of class II antigens are encoded in the MHC. The class II antigens are further divided into DR, DQ, and DP antigens. Class II antigens are expressed on B-cells and monocytes and can be induced on many other cell types following inflammation or injury. The DQ and DP antigens each have polymorphic α - and β -chains, which can dimerize in various combinations.

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 haplotypes (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 one HLA haplotype with their offspring and are considered haploidentical. 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 volunteer donor registries.

For the majority of patients who lack a matched related donor, an HLA-identical unrelated donor represents an alternative stem cell source. Millions of potential donors have been HLA typed and are listed with national and international registries. The utility of these registries has been greatest for patients with common haplotypes for whom, because of linkage disequilibrium, the haplotype is found 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.

Cord blood transplantation and mismatched related donor transplantation

Because of the inability to identify a fully matched related or unrelated donor in a timely fashion, additional sources of stem cells have been explored. It is estimated that only one-third of patients in need of an unrelated donor will be able to find a match. Therefore, umbilical cord blood (UCB) cells harvested from the umbilical cord of newborns represent a rapidly growing source of HSCs. UCB contains hematopoietic progenitors capable of hematopoietic reconstitution, can be obtained within a short time span (available on average in 13.5 days in contrast to a matched unrelated donor search of 3-4 months), and demonstrates less allogeneic reactivity responsible for graft-versus-host disease (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 relative low incidence of GVHD even with two- and three-antigen mismatches.

The greatest limitations of UCB transplantation are the slow rate of engraftment, prolonged myelosuppression, and delayed immune reconstitution, which results in higher rates of death from infection; all of these limitations are related to the relatively low progenitor cell dose in cord blood. This low progenitor cell dose has hampered the ability to obtain rapid engraftment in patients who weigh >50 kg.

A number of trials have been performed to determine the feasibility of UCB transplantation for adults, engraftment generally is slow with a median time to neutrophil engraftment of 3-4 weeks and up to 10% of patients failing to engraft. A more recent analysis from the International Bone Marrow Transplant Registry (IBMTR) 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 speed engraftment have included the use of two cord products in a single patient, supplementation with CD34¹ 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 the volunteer donor registries are mismatched family members. Donors that share one haplotype with the recipient are called haploidentical. Parents are always haploidentical with their children, and siblings have a 50% chance of being haploidentical with each other. It is estimated that approximately 90% of patients will have an available haploidentical donor.

The major challenges associated with haploidentical transplantation are the high rate of 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 post-transplantation chemotherapy with either cyclophosphamide or bortezomib. 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.

Stem cell sources and procurement

HSCs reside primarily in the bone marrow but circulate in the peripheral blood at low levels. Chemotherapy, colony-stimulating factors, and the CXCR4 inhibitor plerixafor can mobilize large quantities of HSCs into the peripheral blood that subsequently can be collected. Initially, allogeneic marrow always was infused fresh after collection with minimal manipulation (filtering of fat globules and bone particles, plasma or red cell reduction depending on ABO incompatibility). With the advent of the cryopreservation agent dimethyl-sulfoxide (DMSO), cryopreservation of autologous marrow 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

HCT initially were obtained exclusively from the marrow cavity under anesthesia with multiple aspirations by a procedure initially described in the 1950. 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, and reduced non-relapse mortality (NRM) with improved survivals.

Peripheral blood

The observation that peripheral blood contained low levels of circulating hematopoietic pluripotent progenitor cells was made initially in the 1970s, but it was not until the cloning and development of colony stimulating factors that large numbers of circulating stem cells could be mobilized into peripheral blood and peripheral blood SCT was made 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. This includes single-agent cytokine, cytokine combinations, and combinations of chemotherapy with cytokines followed by leukapheresis. HSC concentration in the bloodstream usually peaks 4-6 days after initiation of therapy with cytokines alone. When chemotherapy with cytokines 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 CD34¹ content has been used, and many centers initiate HSC collection when CD34¹ cell count exceeds 5-10 cells/mL.

Peripheral blood progenitor cells have almost completely replaced bone marrow as the HSC source for patients undergoing autologous SCT 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 between 2 and 10 million CD34⁺ cells/kg are analyzed; however, no prospective trial looking at CD34⁺ cell dose as a factor in transplant outcomes has ever been performed.

Despite the use of chemotherapy–cytokine combination regimens, mobilization failure still occurs in some patients. Prior treatment is the single most important factor affecting stem cells yields. Prior treatment with stem cell toxins, short interval since last chemotherapy, previous radiation, hypocellular marrow, 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 transplantation evaluation before repeated salvage attempts that may adversely affect stem cell collections.

A novel chemokine, plerixafor (Mozobil; Genzyme, Cambridge, MA), was approved in 2009 as a mobilization agent in combination with G-CSF. Plerixafor, formerly known as AMD3100, 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 I study, it induced modest leukocytosis when administered intravenously to HIV1-infected patients. On the basis of this observation, plerixafor was tested for its ability to mobilize CD34¹ and hematopoietic progenitor cells from marrow to peripheral blood. In a pilot study that included patients with myeloma or lymphoma, plerixafor caused a rapid and statistically significant increase in the total WBC and peripheral blood CD34¹ counts at 4 and 6 hours after a single injection. The results of two phase III randomized studies, one involving lymphoma patients and the other for myeloma patients, recently were reported. 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-plus-plerixafor arm reached the primary end-point compared with the G-CSF-alone arm. Plerixafor was a well-tolerated agent, and the most common adverse events were gastrointestinal disorders and injection site reactions. These results led to approval by the U.S. Food and Drug Administration (FDA) of this agent in 2009. In the autologous transplant setting, peripheral blood SCT has almost totally replaced bone marrow as a source of stem cells because of the ability to collect a large number of stem cells that result in a more rapid engraftment and reduction in complications.

In the setting of allogeneic SCT, peripheral blood SCT is associated with faster neutrophil engraftment but also with higher rates of chronic GVHD. Nine randomized trials have been performed comparing peripheral blood versus 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 3-4 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 risks of chronic GVHD has led to the use of bone marrow as the preferred source of stem cells. Use of T-cell depletion with CD34 selection has been used to reduce the increased risk of chronic GVHD but further prospective trials are needed.

Cord blood

Broxmeyer et al. (1989) were the first to report the presence of HPC's in cord blood using the granulocyte-macrophage progenitor cells (CFU-GM) assay and 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 cord blood banking. This experience led to the first successful cord blood transplant in a young patient with Fanconi anemia.

Conditioning regimens

The combination of chemical and physical agents given before SCT is known as the conditioning or preparative regimen. The purpose of this conditioning regimen in both the autograft and allograft setting is to eradicate the malignancy

exploiting the dose response phenomena that most cancer cells exhibit. In the setting of allogeneic SCT, the conditioning regimen serves a second purpose, which is to suppress the host immune system. The more immunosuppressive the conditioning regimen, the better the chance for engraftment. Conditioning regimen intensity has been classified according to their myelosuppressive effects into myeloablative, reduced intensity, and nonmyeloablative. Table 14-3 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 120-200 mg/kg are combined with radiation in a dose of 8-12 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. Several randomized studies and a meta-analysis have been conducted to compare BuCy with CyTBI. Both regimens are equally efficacious for treatment of acute myeloid leukemia (AML) and chronic myelogenous leukemia (CML). For treatment of patients with ALL, TBI-containing regimens

Table 14-3 Commonly used conditioning regimens.

Allogeneic stem cell transplantation	
Myeloablative conditioning	
CyTBI	Cyclophosphamide 120 mg/kg + TBI 8-12 Gy*
BuCy	Cyclophosphamide 120 mg/kg + busulfan 16 mg/kg PO or IV equivalent
FluBu	Fludarabine 120-150 mg/m ² + busulfan 16 mg/kg PO or IV equivalent
Nonmyeloablative conditioning	
Flu TBI	Fludarabine + TBI 2 Gy
Flu Mel	Fludarabine + melphalan 140 mg/m ²
Flu Cy	Fludarabine + cyclophosphamide 60 mg/kg
Flu Bu	Fludarabine + busulfan 8 mg/kg PO or IV equivalent
Cy ATG	Cyclophosphamide 4 gm/m ² + ATG [†]
Predominantly autologous stem cell transplantation	
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.

ATG = antithymocyte globulin; TBI = total body irradiation.

may be slightly superior. 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 veno-occlusive disease (VOD) and irreversible alopecia. More recently fludarabine/busulfan (FLUBU) combinations have become increasingly more utilized because cyclophosphamide and its metabolites have been implicated in the development of VOD. Busulfan-based protocols generally are recommended in very young children because of the long-term effects of 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 <50–60 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 so-called *nonmyeloablative* or *reduced-intensity conditioning* (RIC) regimens (also called minitransplantation regimens). These regimens often use lower doses of busulfan, melphalan, cyclophosphamide, or TBI (typically 2 Gy) often in combination with fludarabine. Nonmyeloablative regimens more frequently have been used in older patients and in patients with comorbidities. These regimens rely more heavily on immunologic (graft-versus-leukemia [GVL]) effects to induce tumor regression and contain lower doses of drugs with cytoreductive activity. GVHD and infections remain the major causes of NRM.

The characteristics of a reduced-intensity conditioning regimen are that they should be associated with low nonhematologic toxicities and some degree of mixed chimerism early posttransplant, and theoretically, they 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; thioguanine <10 mg/kg; and TBI <500 cGy single fraction or 800 cGy fractionated. These definitions are somewhat arbitrary but are important for retrospective studies.

No randomized trials have been performed comparing transplant outcomes in patients with hematologic malignancies using different regimen intensities (although one is ongoing under the auspices of the Blood and Marrow Transplant Clinical Trials Network[BMT-CTN]). Retrospective comparisons suggest similar outcomes with reduced-intensity conditioning regimens when compared with myeloablative regimens. One Center for International

Blood and Marrow Transplant Research (CIBMTR) analysis, however, did report inferior outcomes for patients with AML receiving low doses of TBI when compared with other reduced-intensity or myeloablative conditioning regimens.

Regimens for autologous stem cell transplantation

Some conditioning regimens are used nearly exclusively for autologous transplantation; they include: (i) carmustine-based regimens, such as carmustine, etoposide, cytarabine, and melphalan or cyclophosphamide, carmustine, and etoposide for large-cell lymphoma or Hodgkin lymphoma; (ii) the high-dose melphalan regimens used for myeloma; and (iii) the ifosfamide, carboplatin, and etoposide (ICE) regimens for lymphoma and germ cell tumors (GCTs).

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. The conditioning regimens for such patients thus 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, although it may need modification in patients with underlying Fanconi syndrome.

Supportive care post stem cell transplantation

Successful SCT requires that the patient tolerate the side effects of the conditioning regimen that the stem cells proliferate and mature adequately as well as treatment and prevention of infectious complications that can occur during the severely immune-compromised state of the patients during the first months after SCT. Most SCTs in North America are performed by specialized teams of physicians, nurses, and other personnel. Outcomes are improved when SCTs are performed by specialized transplant units that perform a minimum of at least 10 transplants a year.

The SCT procedure can be divided in five phases as summarized in Table 14-4.

Table 14-4 Stem cell transplant complications according to transplant phase.

Phase	I-Chemotherapy Phase	II-Cytopenic Phase	III-Early Recovery	IV-Early Convalescence	V-Late Convalescence
Infections	Catheter-related GPC	GPC GNR from GI toxicity HSV Fungal infections	Resistant GNR or GPC Fungal infections CMV reactivation EBV reactivation Other viruses	Viral reactivation Pneumocystis Encapsulated GPC	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		
Hepatic	Transaminitis	Transaminitis Sinusoidal obstruction syndrome Liver GVHD	Transaminitis Sinusoidal obstruction syndrome Liver GVHD	Hepatitis reactivation	Cirrhosis
Cardiac	Arrhythmias (rare) Fluid overload	Hypertension from CNI	Hypertension from CNI		Congestive heart failure Premature coronary vascular disease
Pulmonary	Pneumonitis (rare)	Pneumonia Fluid overload	Idiopathic pneumonia syndrome		Bronchiolitis obliterans Hyperactive airway disease
Neurologic	Seizures from busulfan (rare with prophylaxis)				Cognitive dysfunction-short-term memory loss Impaired concentration
Endocrine	Hyperglycemia	Hyperglycemia from CNI	Hyperglycemia from CNI Increase creatinine Electrolyte disturbances	Hyperglycemia Hypothyroidism Chronic renal failure	Metabolic syndrome
Renal	Increase creatinine Electrolyte abnormalities	Increase creatinine due to drugs (antibiotics, antifungals, CNI) Electrolyte disturbances	Initial presentation can be rash and fevers “Cytokine Storm”	Late acute GVHD presents as acute onset diarrhea or rash	
Acute graft vs. host				Usually presents in the context of immune suppression withdrawal	
Chronic graft vs. host					
Other					Cataracts Secondary malignancies
Timing	D-10 to Day 0	D0 to engraftment usually D+15-30	Engraftment +5-7 days	D+30 to 6-12 months depending on immune reconstitution	12 months post-SCT onward

GPC = gram positive cocci; GNR = gram negative rods; GI = gastrointestinal; HSV = herpes simplex virus; CMV = cytomegalovirus; EBV = Ebstein-Barr virus; GVHD = graft vs. host disease; CNI = calcineurin inhibitor; D = Day; SCT = stem cell transplantation.

Phase I: chemotherapy phase

During this phase, chemotherapy (usually at high doses) with or without radiation is given to the patient to eliminate any residual malignant cells and provide space for the donor stem cells. Phase I finishes with the infusion of the stem cells or bone marrow provided either by the patient in the case of an autologous transplant or by a donor in the case of an allogeneic transplant.

Phase II: cytopenic phase

The most obvious effects of the high doses of chemoradiotherapy are felt during this phase. Severe myelosuppression and disruption of the gastrointestinal mucosa manifested as stomatitis and diarrhea during this period can last 10–28 days. During this period, serious infections and organ toxicities can occur.

Phase III: early recovery phase

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 GVHD can begin to manifest in the allograft setting.

Phase IV: early convalescence phase

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 antibiotic, antiviral, and antifungal prophylaxis as well as close monitoring by the transplant team.

Phase V: late convalescence

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 or recurrence of the original malignancy.

Stem cell transplant complications

Myelosuppression

Severe myelosuppression is a universal complication of myeloablative conditioning regimens regardless of the stem cell source. The duration of the myelosuppression depends on various factors, including stem cell dose, use of methotrexate as GVHD prophylaxis, extent of prior therapy, and stem

cell source. Engraftment is defined as sustained recovery of an absolute neutrophil count (ANC) of 500 neutrophils per liter or more for 3 consecutive days. In the context of an allogeneic SCT, this also implies evidence of donor cell engraftment; in the context of an autologous SCT, neutrophil recovery is synonymous with engraftment. Filgrastim has been shown to reduce the time to neutrophil recovery in both the autologous and allogeneic setting but with no definitive improvement in SCT outcomes.

Graft failure

Graft failure is an unusual but often fatal complication of SCT. 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 disparity of the graft, with T-cell depletion of the graft, 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 SCT 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 postautologous SCT and have a higher rate of developing secondary myelodysplastic syndrome (MDS) or AML.

Infections

Infections are a major cause of life-threatening complications in SCT. Their prevention, diagnosis, and treatment are important components of the care of the SCT patient. Major advances in this area have decreased NRM. Although this is an ever-changing field, the Centers for Disease Control and Prevention (CDC) recommendations published in 2000 and updated in 2009 provide an essential framework for treatment and prevention.

Bacterial infections occur with high frequency during the neutropenic period after transplantation, and guidelines for their prevention and management are similar to those in other neutropenic patients. Many centers use prophylactic quinolone therapy for gut decontamination in patients older than 12 years of age during the neutropenic period. Their use in younger children is controversial secondary to

older safety data in an animal model that restricted the use of quinolones in this age-group. The American Academy of Pediatrics currently recommends that quinolones 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 quinolones 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 their therapy. They are at particular risk for fulminant infections with encapsulated Gram-positive organisms, particularly *Pneumococcus*. They should receive prophylaxis with penicillin V potassium or trimethoprim-sulfamethoxazole.

SCT patients are at high risk for *P. jiroveci*, and prophylaxis is recommended. For those allergic to trimethoprim-sulfamethoxazole, alternatives such as pentamidine or dapsone are routine. 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 and also with immunosuppression and GVHD. Yeast (*Candida*) infections are rare with fluconazole prophylaxis. When such infections occur, they frequently are caused by fluconazole-resistant organisms. Airborne molds, particularly *Aspergillus*, remain a major hazard for patients undergoing allogeneic transplantation, despite the use of high-efficiency particulate air filtration. The azoles (voriconazole and posaconazole) and echinocandins (caspofungin, micafungin, and anidulafungin) with potent activity against molds have improved the outcome for such patients. Concerns with new azoles include their toxicity profile (neurologic and hepatic toxicity), which can be life threatening. Interactions with the metabolism of calcineurin inhibitors warrant the need for careful monitoring and often dose reduction of tacrolimus and cyclosporine. Also, because *Aspergillus* is treated more successfully, cases of mucormycosis increasingly are reported and necessitate treatment with amphotericin derivatives or posaconazole.

Viral infections are common after SCT. Cytomegalovirus (CMV) infection used to be a major cause of pneumonia and death in SCT recipients. CMV infection post-SCT usually occurs as a consequence of CMV reactivation in patients previously exposed to CMV as indicated by positive antibody titers (CMV⁺ patients). The incidence of reactivation ranges from 40% to 60% in the allogeneic setting and less than 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 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 are not readily available. Fortunately, 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 SCT. Ganciclovir, oral valganciclovir, high-dose acyclovir, or valacyclovir have all been used for prophylaxis of CMV reactivation in patients at high risk. 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.

For patients who develop CMV viremia, preemptive treatment with ganciclovir or valganciclovir is initiated immediately. This strategy of preemptive treatment has significantly decreased the occurrence of CMV disease in the early months after transplantation. Oral valganciclovir (but not oral ganciclovir) is a convenient and effective oral alternative for preemptive and prophylactic treatment. Alternative medications for preemptive treatment include foscarnet (equally efficacious but more nephrotoxic) and cidofovir (requires only once-weekly administration but is less extensively tested and much more 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), Epstein-Barr virus (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. Treatment with rituximab is typically first-line

therapy. HHV-6 is a cause of posttransplantation encephalitis and aplasia. Others have postulated a link with interstitial pneumonia.

Adenovirus has been the cause of 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 (RSV), and influenza can lead to fatal pneumonias. Some centers have recommended screening of all patients during RSV 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

TBI frequently is associated with generalized erythema followed by hyperpigmentation. Thiotepa 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 thiotepa skin toxicity. Likewise, patients receiving thiotepa 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 tissue the gastrointestinal tract is the single most commonly affected organ by the conditioning regimen. As a rapidly dividing tissue that is being constantly regenerated, the toxic effects of the conditioning regimen are felt all throughout the gastrointestinal tract. The most common manifestations of gastrointestinal toxicity are nausea, vomiting, oral lesions, esophagitis, and diarrhea.

Carmustine, TBI, and cyclophosphamide are highly emetogenic agents, whereas melphalan and busulfan are classified as moderately so. Thus, adequate control of nausea and vomiting requires prophylaxis as well as frequent use of breakthrough medications. Acute emesis usually involves combination therapy with corticosteroids and 5-hydroxytryptamine-3 receptor antagonists. Despite this, complete control of nausea and vomiting (no nausea, no emesis, and no need for breakthrough medications) is achieved in less than 20% of the population. Destruction of the oral and gastrointestinal mucosa is a significant dose-limiting complication of high-dose therapy regimens.

Stomatitis refers to the painful ulcerations and sores that occur on the mouth, lips, gums, and throat of patients usually 5-7 days after conditioning and can be seen in up to 90% of SCT recipients. The most important risk factor for developing severe oral mucositis is the intensity of the conditioning regimen; other factors that predict development of severe oral mucositis are poor oral hygiene, extensive prior therapy, and concurrent chemo-radiation. Oral mucositis is a significant cause of morbidity post SCT. Studies have shown that the incidence of severe oral mucositis post high-dose therapy can be reduced by palifermin in the setting of TBI and by using ice chips during chemotherapy infusion in the setting of high-dose melphalan and by amifostine. Once oral mucositis occurs, treatment is primarily supportive with intravenous hydration and 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 endoscopic evaluation for tissue procurement to rule out GVHD and other treatable causes. Treatment is supportive and symptomatic.

Hepatic complications

VOD or sinusoidal obstruction syndrome (SOS) is one of the most common and lethal toxicities of SCT; it occurs in 10%-60% of patients receiving transplants, depending on both the risk factors for the patients and the vigor with which the diagnosis is pursued. 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 (TNF α) predict development of VOD.

VOD is more common in patients with evidence of prior hepatocellular damage at the time of transplantation, heavy pretreatment before SCT, prolonged and elevated busulfan levels, or >10-12 Gy TBI. Other drugs such as nitrosoureas (carmustine) also have been implicated in VOD/SOS. Studies suggest that prior exposure to gemtuzumab 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 VOD/SOS but remain controversial.

The clinical diagnosis of VOD usually required weight gain or ascites, tender hepatomegaly, and jaundice. 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 agent.

Recently, defibrotide, a polydeoxyribonucleotide, has shown encouraging results in patients with established VOD. 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 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 VOD was seen in 36%. Younger patients, those receiving autologous SCT, and those with abnormal portal flow had the highest response rates. Defibrotide is not approved by the FDA, although the results of a recently completed phase III trial are eagerly awaited.

Pulmonary toxicities

Pulmonary complications occur frequently after SCT and are associated with a high mortality. To stratify transplantation patients into different risk categories, pretransplantation evaluation often includes a detailed pulmonary function test (PFT) and two-dimensional echocardiogram or radionuclide ventriculography. The utility of these tests, however, is doubtful. In a retrospective study from the Fred Hutchinson Cancer Research Center that included 1,297 patients, decreased diffusing capacity of the lung for carbon monoxide and elevated alveolar-arterial partial pressure of oxygen were predictors for increased mortality. Most transplantation centers, however, will not exclude a patient from transplantation based solely on an abnormal pre-SCT PFT. Similarly, baseline reduced left-ventricular ejection fraction predicted for cardiac toxicity after SCT but failed to predict life-threatening events.

During the early posttransplantation period (days 0-30), regimen-related toxicity and infectious etiologies account for most of the pulmonary events. Although most focal infiltrates are infectious in origin, diffuse infiltrates related to regimen-related toxicity also should be considered. The differential diagnosis of diffuse infiltrates during early post-SCT includes iatrogenic fluid overload, pulmonary edema (cardiogenic and noncardiogenic), idiopathic pneumonia syndrome (IPS), adult respiratory distress syndrome from

chemoradiotherapy injury or sepsis, and diffuse alveolar hemorrhage (DAH). Cardiogenic pulmonary edema and septicemia 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 30-150, but its incidence has decreased dramatically with the use of preemptive treatment strategies for the prevention of CMV disease. During this period, opportunistic and idiopathic pneumonias dominate the pulmonary complications. It takes approximately 3-6 months for the immune function of patients undergoing SCT to return to normal and longer for patients who suffer from chronic GVHD. Infectious etiologies during this phase include bacteria, fungi, viruses, *Nocardia*, mycobacteria, and *P. jiroveci*. Furthermore, approximately 10% of patients with chronic GVHD develop bronchiolitis obliterans, a severe obstructive airflow disease.

Idiopathic pneumonia syndrome

IPS is a condition characterized by diffuse alveolar injury with fever, cough, dyspnea, hypoxemia, and restrictive physiology. Chest x-ray usually demonstrates multilobular pulmonary infiltrates. This is a diagnosis of exclusion. BAL must be negative for infectious etiologies, including bacteria, fungi, 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. Treatment of IPS is mostly supportive, but high-dose corticosteroids often are given. They may be beneficial in patients in whom pulmonary damage is due to carmustine or in those with the closely related syndrome of DAH.

Diffuse alveolar hemorrhage

DAH occurs most commonly in the first weeks after SCT and presents as idiopathic pneumonia with or without hemoptysis. 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 2-3 days, and negative microbiologic studies. Treatment of DAH is largely supportive, but retrospective studies suggest that high-dose corticosteroids with a starting dose in the range of 1 g/d are often beneficial.

Transplantation-related obstructive airway disease

Approximately 6%-10% patients with chronic GVHD develop chronic airway obstruction. The most common

histologic finding is constrictive bronchiolitis obliterans. Bronchiolitis obliterans typically presents 3-12 months after an allogeneic SCT with gradual onset of dyspnea, dry cough associated with occasional wheezing, and inspiratory crackles. PFTs demonstrate an obstructive pattern that does not respond to bronchodilator therapy and reduced diffusing capacity of the lung for carbon monoxide. 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 effective treatment of patients with bronchiolitis obliterans, and current treatment mostly is directed at the underlying chronic GVHD with immunosuppressive therapy. Lung transplantation offers some promise.

Thrombotic microangiopathy

Posttransplantation thrombotic microangiopathy (TMA) presents as a spectrum of disease, ranging from mild microangiopathic anemia to thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome and occurs more commonly after allogeneic and unrelated donor SCT. TTP frequently presents with fever, neurologic symptoms, microangiopathic hemolytic anemia, thrombocytopenia, and renal impairment. In children, TMA more closely resembles hemolytic uremic syndrome. In some patients, TMA appears to be related to cyclosporine nephrotoxicity and responds to discontinuing the cyclosporine. But other patients have a fulminant course and a very high mortality rate. Autopsy findings include arteriolar thrombosis in the kidneys. In many patients, fungal infection, sepsis, or severe acute GVHD appear to be underlying the microangiopathic processes. Unlike patients with idiopathic TTP, patients with transplantation-related TTP do not respond to plasma exchange. TTP outside the transplantation setting has been associated with immunoglobulin G (IgG) antibodies that inhibit the cleaving protease of von Willebrand factor in the plasma. No such mechanism was found in cases of posttransplantation TTP, pointing to another as-yet-unidentified cause for this syndrome.

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 toxicity to the bladder from cyclophosphamide metabolites. Late-onset hemorrhagic cystitis often is associated with viral infection with BK virus and occasionally with adenovirus. Hemorrhagic cystitis can be severe and can require continuous bladder irrigation, diverting nephrostomy tubes, and occasionally

formalin instillation until the bladder heals. As mentioned, pulmonary hemorrhage can be a serious complication of transplantation and most often is attributed to preparative regimen toxicity.

Iron overload

Iron overload has been identified to be an adverse prognostic factor for children with thalassemia undergoing SCT, and there is an increasing evidence that iron overload also may have deleterious effects for patients with hematologic malignancies who undergo SCT. This particular patient population often is transfused heavily before SCT and continues to require transfusions in the peritransplantation period. One red blood cell unit contains 200-250 mg of iron, and significant iron accumulation can occur after 10-20 RBC transfusions. Iron overload increases the risk of infections, VOD, and hepatic dysfunction.

Graft-versus-host disease

Acute and chronic GVHD traditionally were 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 two subcategories of chronic GVHD (classic and overlap acute or chronic) are recognized.

Acute GVHD

Acute GVHD is characterized in its mildest forms by skin rash. As the disease worsens, the confluent rash may progress to blistering of the skin similar to a severe burn, profound diarrhea with crampy abdominal pain, and hepatic dysfunction with marked hyperbilirubinemia. Acute GVHD is graded by the extent of skin rash, the amount of diarrhea, and the 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 grave prognosis, with mortality rates >90%.

Acute GVHD was first considered a “pure” T-cell-mediated disease with cellular injury thought to be the result

of infiltration of T-effector 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 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 more cytokines that further activate additional donor T-cells and other inflammatory cells, including macrophages induced to 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 may decrease GVHD by interrupting this cascade. Of particular interest will be the further elucidation of the role of CD4¹ 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. Recent data have shown that allogeneic PBSC transplantation is 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.

Prophylaxis of GVHD has been more successful than treatment. The most commonly used prophylaxis regimens combine a calcineurin inhibitor (cyclosporine or tacrolimus) with methotrexate. Because of the renal and mucosal toxicities seen with these regimens alternative prophylactic regimens are being explored. Sirolimus and mycophenolate mofetil now commonly are used as alternatives to methotrexate to decrease the toxicity of GVHD prophylaxis.

Other methods to prevent GVHD include depleting the graft of donor T-cells either by an in vitro procedure after procurement of the stem 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 higher infection rates because of delayed immune reconstitution.

Therapy for acute GVHD consists of high-dose corticosteroids, typically 2 mg/kg/d, which are tapered upon obtaining a response. Calcineurin inhibitors will be continued or restarted. Patients not responding to or experiencing recurrence on high doses of corticosteroids (considered steroid refractory) have a poor prognosis from continued acute GVHD, infection, and chronic GVHD. Other agents added in the steroid-refractory setting include mycophenolate

mofetil, pentostatin, ATG, and monoclonal antibodies, such as infliximab, etanercept, and rituximab. The responses are low, however, and patients typically succumb to opportunistic infection in the setting of profound immunosuppression.

Chronic GVHD

Chronic GVHD affects from 40% to 80% of long-term-survivors of allogeneic SCT. 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 recent 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.

Many of the 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 14-5. Diagnostic features of chronic GVHD typically involve the skin and mucosa. They 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 an esophageal web and strictures, fasciitis, and joint contractures. Finally, bronchiolitis obliterans 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 new proposal, 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

Table 14-5 Signs and symptoms of chronic graft-versus-host disease (GVHD).

Organ or site	Diagnostic (sufficient to establish the diagnosis of chronic GVHD)	Distinctive (seen in chronic GVHD, but insufficient alone to establish a diagnosis of chronic GVHD)	Other features*	Common (seen with both acute and chronic GVHD)
Skin	Poikiloderma Lichen planus-like features Sclerotic features Morphea-like features Lichen scleroses-like features	Depigmentation	Sweat impairment Ichthyosis keratosis pilaris hypopigmentation Hyperpigmentation	Erythema maculopapular rash pruritus
Nails		Dystrophy Longitudinal ridging, splitting, or brittle features onycholysis Pterygium unguis Nail loss (usually symmetric; affects most nails) [†]		
Scalp and body hair		New onset of scarring or nonscarring scalp alopecia (after recover from chemoradiotherapy)	Thinning scalp hair, typically patchy, scarce, or dull (not explained by endocrine or other causes) premature gray hair	
Mouth	Lichen-type features Hyperkeratotic plaques Restriction of mouth opening from sclerosis	Xerostomia mucocele mucosal atrophy pseudomembranes [†] Ulcers [†]		Gingivitis mucositis Erythema pain
Eyes		New onset dry, gritty, or painful eyes [†] Cicatricial conjunctivitis Keratoconjunctivitis sicca [†] Confluent areas of punctate keratopathy	Photophobia periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	
Genitalia	Lichen planus-like features 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 Failure to thrive (infants and children)
Liver				Total bilirubin, alkaline phosphatase .2–3 upper limit of normal [†] ALT or AST .2–3 upper limit of normal [†] BOOP
Lung	BO diagnosed with lung biopsy [‡]	BO diagnosed with PFTs and radiology [†]		
Muscles, fascia, joints	Fasciitis Joints stiffness or contractures secondary to sclerosis	Myositis or polymyositis [†]	Edema Muscle cramps Arthralgia or arthritis	

(continued)

Table 14-5 Signs and symptoms of chronic graft-versus-host disease (GVHD) (continued)

Organ of site	Diagnostic (sufficient to establish the diagnosis of chronic GVHD)	Distinctive (seen in chronic GVHD, but insufficient alone to establish diagnosis of chronic GVHD)	Other features*	Common (seen with both acute and chronic GVHD)
Hematopoietic and immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hypergammaglobulinemia Autoantibodies (AIHA and ITP) Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis cardiac conduction abnormality or cardiomyopathy	
Other				

*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).

From Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.

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 under evaluation include psoralen plus ultraviolet A, extracorporeal photopheresis, pentostatin, imatinib, and rituximab.

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 organisms 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. jiroveci* pneumonia (formerly known as *Pneumocystis carinii*), and fungal infections (yeast and mold).

Late effects

As the number of long-term survivors following a transplantation increases and many of these individuals have returned

to their referring physicians, the need for understanding and continued follow-up is essential both for the care of the survivors and to anticipate the needs of the group as a whole. The joint recommendations of the European Bone Marrow Transplantation (EBMT) Group, the CIBMTR, and the American Society of Blood and Marrow Transplantation were recently published. These recommendations are based in part on published data and in part on common practice. Careful evaluation on an annual basis is recommended with close monitoring and preventive screening, especially for the problems discussed in this chapter. Many of the late complications seen after SCT are especially profound for younger patients.

Endocrine adverse effects

Endocrine sequelae of myeloablative transplantation have been well documented but may be underappreciated. Children should be followed to ensure that adequate growth is obtained through adolescence. After conditioning with CyTBI, 20%-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 the transplantation.

The prevalence of damage to the gonadal tissue is high and may result in delay or absence of development of secondary sexual characteristics and the need for hormonal

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, the high risk of infertility after SCT is a major issue, and counseling for sperm or egg banking should be considered for such young patients before SCT. Recent evaluation of 39 male patients two years following SCT at a single institution demonstrated spermatogenesis in 28% of the patients. Those more likely to have sperm included men >25 years of age at transplantation, men with a longer interval from transplantation, and men who had not had chronic GVHD. Unfortunately, although sperm banking is readily available, currently, only fertilized eggs are readily stored. Research is ongoing into the cryopreservation of unfertilized eggs or ovaries. For many patients, the course of their disease does not allow this luxury; however, counseling with fertility specialists after the procedure, in the future, may allow new options.

Musculoskeletal complications

Patients receiving high-dose corticosteroids for their disease or for GVHD have an increased risk of developing avascular joint necrosis and myopathies. In addition, loss of range of motion may be seen in patients with a history of chronic GVHD even if the disease is controlled. Osteoporosis resulting from steroid use and therapy-induced menopause is common. All patients should obtain a bone densitometry at 1 year after transplantation.

Psychosocial considerations

The long-term cognitive effects of prior therapy and SCT continue to be evaluated. Significant central nervous system (CNS) toxicity has been seen, especially in young patients receiving intensive intrathecal chemotherapy or CNS radiotherapy before transplantation. Previous evaluations involving quality-of-life (QOL) assessments completed by parents appear to underestimate the child's QOL 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 adults who are finding tasks at home and work more difficult after transplantation. The loss of executive function after therapy has not been evaluated. 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.

Second malignancies and posttransplantation lymphoproliferative disorders

Survivors of allogeneic transplantation are at increased risk for a variety of second malignancies, including a two- to threefold 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. Melanoma and basal cell carcinoma are common in patients, especially those with chronic GVHD. Patients should be instructed to avoid ultraviolet exposures and to use sunscreens and protective clothing.

Posttransplantation lymphoproliferative disorders after allogeneic transplantation are usually related to EBV. They occur more commonly after T-cell-depleted transplantation or in other profoundly immunosuppressed states. Treatment consists of decreasing immunosuppression, monoclonal antibody therapy (in particular rituximab), donor leukocyte infusions (DLIs), and sometimes chemotherapy.

Long-term survivors of autologous transplantation are at considerable risk for therapy-related leukemia. In some series, the cumulative incidence exceeds 10%. The risk is increased with high-dose TBI used for conditioning, is related to the type and intensity of chemotherapy received before transplantation, and possibly is 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 were detected in the marrow or stem cell product of patients destined to develop therapy-related MDS, further implicating pretransplantation chemotherapy.

Relapse and the graft-versus-malignancy effect

Relapse remains the most important cause of treatment failure in both the autologous and the allogeneic transplant setting. The mechanisms underlying disease recurrence are poorly understood. In the setting of autologous SCT, the existence of cancer stem cells that may be quiescent and therefore impervious to the effects of high-dose chemotherapy and radiation has been postulated. The potential benefit of prolonged posttransplant therapy (such as lenalidomide) supports this concept.

In contrast to autologous SCT, allogeneic SCT is associated with a GVT effect mediated by alloreactive donor T- and B-cells that provide an inherent posttransplant immune surveillance mechanism. The importance of GVT initially was studied by comparing relapse rates between syngeneic and allogeneic transplantation 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 CML in chronic phase had an increased rate of recurrence after syngeneic transplantation. Relapse rates after syngeneic transplantation for lymphoma or for *acute lymphoblastic leukemia* (ALL) in CR1 are not increased compared with those after allogeneic transplantation. Definitive evidence for a GVT effect comes from the use of DLIs. DLI confers a direct graft-versus-malignancy effect by infusion of alloreactive donor lymphocytes. Purposes of DLI include conversion of mixed-donor chimerism to full-donor chimerism after SCT as preemptive therapy to prevent relapse or for the treatment of relapse.

The mechanisms of relapse after allogeneic SCT have not been well studied. These malignancies have not only escaped the effects of high dose alkylating agents, but have also evolved mechanisms to overcome the immune mediated graft vs. tumor effects. Clonal evolution, loss of specific surface antigens, or development of local immune suppressive mechanisms have all been postulated.

Treatment and prevention of relapse after allogeneic SCT remains a major challenge. Only recently, the use of hypomethylating agents has been shown to potentially reduce the risk of relapse in patients with AML and MDS, and a phase III trial is under way.

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%-10%, with acute GVHD and marrow aplasia being the leading causes of death. The incidence of both acute and chronic GVHD after DLI is ~40%-60%, with more than half of the patients who develop chronic GVHD having extensive disease. The onset of acute GVHD typically occurs 32-42 days after DLI.

Posttransplant cellular therapies as a strategy for relapse prevention continues 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.

Transplantation for specific diseases

The task of mastering the roles and results for transplantation is daunting: There are various types of stem cell source (autologous, allogeneic); many diseases; and for each disease,

different states of aggression (remission, relapse). Things can be simplified, however, into some basic rules of thumb:

1. Risk groups fall into four categories, based on disease (low, intermediate, high) and stage (low for cases remission vs. high for relapse or refractory). Thus, patients with low disease risk and low stage have a low overall risk (4 year OS 64%); patients with low disease and high stage, or intermediate risk and low stage, rate an intermediate overall risks (OS 46%); patients with intermediate disease and high stage, or high disease and low stage, have a high overall risk (OS 26%); and, last, high disease and high stage give a very high overall risk (OS 6%).
2. For the vast majority of cases, different allogeneic sources of stem cells give similar results. Thus, outcomes after matched related, matched unrelated, cord, and haploid-identical transplants appear similar. The differences in these approaches are from the causes of failure (eg, relapse in matched related donor [MRD], GVHD in matched unrelated donor [MUD], infections in cord transplants).
3. In diseases amenable to a nonmyeloablative approach, results from “full” ablative and RIC are quite similar. Relapses are more common in the RIC setting, but this is offset by less NRM.

Acute myelogenous leukemia

Success of chemotherapeutic regimens in AML is largely dictated by cytogenetic risk and age. Chemotherapy is potentially curative for APL and core-binding factor (CBF) leukemia (good-risk cytogenetics). Patients with poor-risk cytogenetics should be offered transplant in CR1, and patients with intermediate risk should entertain the idea, as a met-analysis of 24 trials and 6,007 patients suggested a benefit in both poor and intermediate risk groups. Of note, molecular studies may push patients up or down the risk group ladder. Thus, intermediate-risk patients with an NPM1 mutation (and no FLT3 mutation) behave like good risk cases; patients with a FLT3 mutation tend to have very high relapse rates. In addition, core binding factor (CBF) cases with a CEBP- α mutation are demoted to the intermediate-risk category. Of note is that “elderly” AML patients (currently ~65 years or greater, although this changes with the age of the investigator) have terrible outcomes with chemotherapy alone, and thus are candidates for an RIC transplant if their disease can be placed into a remission. Results for transplantation in CR1 are predictably better than cases that relapse, are in CR2, or have refractory AML. Thus, survival in CR1 is approximately 40%-60%, whereas CR2 rates are ~25% and refractory rates are ~10%. This data should not be misinterpreted to suggest that patients should take a chance on chemotherapy, and if not cured, get the same outcome as upfront transplantation

by delaying until CR2. The result data on CR2 are highly biased, as it accounts for only a fraction of patients who managed to escape death by leukemia or infection, and who actually obtain a remission.

The standard preparative regimen used TBI and Cytoxan, but a randomized trial showed that a busulfan and Cytoxan regimen gave similar results, so this has become the standard in many centers. Studies with “targeted” radiotherapy, whereby I¹³¹ is linked to an antibody to CD45, shows promising results. The approach is limited, however, because special “safe” rooms must be used to limit radiation penetration to innocent bystanders and because of limitations to antibody-radioisotope production. As noted, the encouraging of RIC regimens has brought this from being only a treatment for the infirm and elderly to being more widely used (indeed, there is currently a nationwide randomized trial for full ablative vs. RIC transplantation for CR1 patients). 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 preferred as the stem cell source, equal results can be obtained with a fully matched–unrelated donor. Moreover, it appears that UCB transplants afford similar results as MRD or MUD transplants. Because most cord units have slow engraftment in adults given the relatively small stem cell dose, many centers now use two cords, which speeds engraftment considerably. A major advantage to a UCB is speed; thus, although a usual unrelated donor search may take 3-4 months, cord units generally are available in a fraction of time. Allogeneic SCT using related haploidentical donors is still investigational, with success mostly limited to patients who have myeloid malignancies in remission at the time of SCT. When cord blood units or haploidentical donors are used, opportunistic infections stemming from delayed immune reconstitution represent a major cause of NRM.

Autologous transplantation for AML has been explored as consolidation for patients in CR1 or CR2. Patients undergoing autologous transplantation in CR1 have a decreased rate of recurrence compared with those receiving conventional chemotherapy but a higher recurrence rate than patients undergoing allogeneic transplantation. There is controversy whether the higher relapse rates in autologous compared with allogeneic transplantation are from contaminated AML cells in the stem cell product or are from the lack of a GVL effect. Either way, the procedure is used only uncommonly, given that most patients who need a transplant have a allogeneic, unrelated, UCB, or haploidentical donor possible.

In AML, sensitive flow cytometry techniques have been used to measure so-called MRD in patients who are in morphological remission. These methods can detect as low as 1 AML cell in a background of 10000 normal cells. Patients with residual disease are at a higher risk of relapse compared

with those without residual disease; thus, it is tempting to use MRD as a guide to suggest which patients should undergo transplant in remission. Studies have shown that patients with MRD at the time of transplant do far worse than those without MRD, with posttransplant relapse rates of 65% versus 18%, respectively.

Acute lymphoblastic leukemia

The role of allogeneic SCT in ALL differs greatly between the pediatric and adult population. The prognosis of pediatric patients with ALL is very good with standard therapy (>80% survival). SCT in first remission is thus limited to the very high-risk population, including children with t(9;22), those whose blasts are hypodiploid, those who have an *MLL* rearrangement [t(4;11)] with a slow response to therapy, and those who fail to obtain a remission after induction therapy. In a recent retrospective study, children with t(9;22) translocation achieved a 65% long-term event-free survival (EFS) after SCT 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 SCT 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.

In adults, allogeneic transplantation in CR1 generally has been reserved for those with high-risk features of ALL. High risk often is defined by the following characteristics: poor-risk cytogenetics, such as t(9;22), t(4;11), t(1;19), and t(8;14); complex karyotype (ie, >5 abnormalities); WBC >30,000/mL with the B-cell phenotype; WBC >100,000/mL with the T-cell phenotype; requiring >4 weeks to achieve complete remission (CR); or age >30-35 years. Approximately 40%-60% of patients who receive transplantation in first remission become long-term survivors. For patients with standard-risk ALL, several recent publications 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 MRCUKALLXII-ECOG2993 trial, which accrued nearly 2,000 newly diagnosed ALL patients from 1993 to 2006. Of the 1,031 Philadelphia chromosome-negative patients who obtained CR with frontline therapy, the 5-year OS among patients who had a donor versus patients without a donor (and who received autologous SCT or chemotherapy) was 53% versus 45%, respectively. The standard-risk patients with a donor had a superior OS compared with patients without a donor. In addition, a recent study of 288 adult ALL patients, classified as either donor available (matched related donor ready and willing) or no donor (no donor, or donor not able or willing) showed a significantly superior survival

(61% vs. 47%), DFS (61% vs. 47%), and relapse rate (24% vs. 55%) in the donor versus no donor group. Lastly, a meta-analysis of >1,200 ALL patients in seven studies showed a higher survival in the patients with a donor compared with the no-donor group.

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), only 10% achieve long-term EFS with standard chemotherapy. A retrospective review found that children with relapsed ALL had better EFS with allogeneic SCT than with chemotherapy alone for early relapse and that, in this population, a TBI-based regimen was superior. The role of SCT in later relapses is debatable due to relatively good results with standard chemotherapy alone. In marked contrast, survival of adult patients with ALL who relapse after chemotherapy is only 5%-10%. If patients can be placed into a second remission, survival rates following allogeneic transplant are between 20% and 25%.

The role of transplantation in Ph+ ALL deserves a special note. The presence of Ph has long been considered a “high-risk” feature, but the advent of tyrosine kinase inhibitors (TKIs), especially when combined with intensive chemotherapy regimens, may be changing this model. Because long-term data with TKI are not yet available, the conservative approach is to offer patients with Ph+ ALL a transplant in CR1.

In both pediatric and adult ALL, the detection of MRD by flow cytometry or by PCR of clonal TCR or IgH gene rearrangements is highly predictive of subsequent relapse. It also is clear, however, that patients with detectable residual disease at the time of transplant have inferior results (due to high relapse rates) compared with patients free of disease.

Chronic myelogenous leukemia

Before the introduction of the TKIs, such as imatinib, dasatinib, and nilotinib, allogeneic SCT was part of standard therapy for patients with CML. Like all therapies for CML, the efficacy of treatment varies with the stage of disease. Thus, for patients with chronic-phase CML undergoing myeloablative allogeneic SCT, the 10-year OS rate was between roughly 70% and 80%, whereas it was only 30%-40% in the accelerated phase and ~10% in blastic phase CML. Similar results have been obtained from patients receiving a transplantation from HLA-identical unrelated donors. Pretransplant variables can define a prognostic scoring system for transplantation in CML. The system devised by Gratwohl uses HLA matching, stage, age, sex of donor and recipient, and time from diagnosis to transplant. The scoring system is effective in defining posttransplant outcomes following an

ablative transplant, with EBMT data showing 70% survival for the best score and 20% survival for the worst score.

The advent of TKIs changed the treatment course in chronic phase CML. To summarize the salient points of TKI therapy: (i) Primary therapy for chronic phase is highly effective, and can produce a CCyR in ~80% of cases. For these patients, survival at 7+ years is nearly 90%. Approximately 20%-30% of cases will fail primary therapy, however, either from intolerance, relapse, or progression to advanced phase disease. (ii) For patients receiving secondary therapy for resistant disease, approximately 50% will achieve a CCyR. The survival for these patients is ~80% at 3 years. Those who do not achieve and maintain a CCyR often relapse with new mutations. (iii) Patients with AP or BC can achieve a CCyR with TKI therapy, but this does not appear to be associated with long-term progression-free survival (PFS). (iv) Patients who have a T315I mutation do not respond to currently available TKI therapy. As natural selection would dictate, selection of this mutation increases as patients become resistant to more and more TKIs.

So which CML patients should be considered for allogeneic SCT? For chronic phase patients, the initial therapy should be a TKI (the considerable debate as to which TKI is outside the boundaries of this review). Both the National Comprehensive Cancer Network and the European Leukemia Network (ELN) have (very similar) guidelines for monitoring response. Using these criteria, roughly 20% of cases will become resistant to primary therapy. For those cases that become resistant to imatinib, roughly 40% will achieve a CCyR with a second-generation TKI, and some of these cases eventually will relapse. In general, natural selection enriches the relapsed population to a higher percentage of the T315I mutation, which is resistant to imatinib, nilotinib, and dasatinib. Thus, transplantation for chronic phase patients can be considered in the rare cases of intolerance to all TKI, and for those who acquire a T315I mutation. Because only a percentage of patients who become resistant become controlled with a second line agent, however, it is wise to begin the search for a donor when “salvage” therapy is started.

Efforts have been made to identify clinical or laboratory variables that will predict outcome after the initiation of secondary TKI therapy. At present, early cytogenetic response to a secondary TKI seems to do well. Thus, patients who are placed on a secondary TKI, but who fail to have any cytogenetic response after 3 months of therapy is unlikely to ever achieve a CCyR. Likewise, failure to achieve at least a minor cytogenetic response by 6 months, or an MCyR by 12 months of secondary therapy, bodes poorly for sustained disease control. Although TKI therapy, by itself or in combination with chemotherapy, can yield responses in advanced-phase CML, these regimens are not curative, and thus, patients who

present or progress to accelerated or blast phase should be considered for transplantation.

The care of the pediatric patient with CML is evolving with the development of imatinib. Related and unrelated SCT in pediatric patients with this disease results in EFS rates of 60%-75%. The lack of long-term data for new agents makes a definitive recommendation difficult. Most centers will begin the child or adolescent on imatinib, while initiating HLA typing and donor search. If there is a good match, the child may proceed to SCT. As the data on imatinib and other similar agents mature, the role in the treatment of childhood CML should be investigated within clinical trials, including close observation for late effects of this novel therapy.

In the pre-TKI era, several studies showed that prior therapy with busulfan or interferon before transplant was associated with poorer outcomes. This appears not 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 pretransplant imatinib. In addition, there is no evidence that resistant patients with Abl mutations have a poorer outcome following transplantation.

Last, CML was one of the early best examples of using a molecular test (RT-PCR of the BCR-ABL mRNA) to predict subsequent relapse following transplantation. Several studies have used TKI therapy (mostly imatinib) 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

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in North America and Europe. Although this disease usually follows an indolent course, it remains incurable with standard therapy, and once patients become fludarabine-refractory, the median survival is ~12 months. Thus, SCT is appropriate for poor-risk CLL, broadly defined as cases with primary resistance to purine analogue-containing therapy or who relapse within 24 months of therapy; cases with biological predictors of aggressive disease, such as ZAP-70, high CD38 expression, and especially mutations/deletion of 17p14(p53); cases with Richter’s transformation; or cases of relapse after an autologous transplant.

The rise of RIC transplantation 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 ages of this population. A number of studies of RIC transplants 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, 4- and 5-year data suggest NRM of ~20%, PFS of ~40%, and OS of 40%-60%. Of note, two studies have shown that unlike chemotherapy, patients with the p53 mutations or ZAP70 risk factors fare no differently following RIC. Not surprisingly, patients who are transplanted with chemosensitive disease, or 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.

High-dose chemotherapy with autologous SCT confers high response rates in CLL and reported remission durations lasting up to 5-6 years. A recent randomized study from the European intergroup compared autografting with observation responding patients after first- or second-line therapy. Autologous transplant was associated with reduced relapse rates compared with observation (54% vs. 76%, respectively), but the OS at 5 years were nearly identical. Moreover, autologous SCT has been associated with posttransplantation MDS/AML (with an incidence as high as 12% reported in one series).

Of note is that umbilical SCTs have been performed on a limited number of CLL cases (<20); like other diseases, it appears that UCB transplantation has similar outcomes to unrelated donor transplants, and thus may be appropriate in cases in which a matched unrelated donor is difficult to obtain.

Myelodysplasia

Allogeneic transplantation is the only curative therapy for MDS. There is no role for autologous transplantation given that a “normal” hematopoietic stem cell may not exist in MDS. Through the evolution of transplantation regimens, a few general rules apply: (i) results are better for early rather than late-stage disease (defined by any of the myriad of classification schemes); (ii) results are worse with poor-risk cytogenetics, or if the patient has secondary MDS that arises subsequent to prior therapy; (iii) matched related and unrelated transplantation yield similar results; and (iv) fully ablative and RIC regimens offer similar results. The major caveat with the last statement, however, is that RIC requires patients to have low aberrant blast counts, so the comparison is valid only in a relatively early stage (or treated into remission) MDS.

Like many diseases, transplantation success is better for cases with early disease. Thus, cases with refractory anemia (RA) have a disease-free survival exceeding 50% (indeed, this may exceed 70% for International Prognostic Symptom Score [IPSS] 0, and 60% for IPSS 0.5-1). In contrast, patients with advanced MDS or secondary MDS have survivals closer to 25%. The differences in DFS mostly are accounted for by

relapse risk, low in early stage disease (~10% or less), and sadly common in advanced disease (>40%). As in AML, cytogenetic risk groups largely map to outcome, again principally dictated by relapse rates after transplantation. The IPSS risk group classification (see previous chapter) does a fine job in risk classification, but a newer five-group classification system, which adds in the monosomal karyotype (see Chapter 18 for definition) looks to be better, especially in defining a very poor risk group (those having more than three clonal cytogenetic abnormalities), for whom survival was <10%.

In some retrospective analysis, age was not associated significantly with outcome, whereas in other studies, advancing age is a poor risk factor. This is likely because the age cutoffs for these various studies are different; recent analysis that include transplants >70 years of age suggest (logically) that at some point, age must matter to transplant results.

There is considerable debate in regards to when a transplant should be performed. A Markov model examined three 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 Int-2 and high-risk disease, but the wait-until-progression option was the optimal strategy for early disease. Models are slaves to their assumptions; a more recent study of nearly 400 patients with ablative and RIC transplants showed that increasing age and a time from diagnosis of >12 months were associated with an inferior result.

In early MDS, the obvious risk is that waiting for disease to advance is accompanied by heavy baggage: (i) higher relapse rates at transplantation and (ii) the potential that the disease will not be controlled by the time transplantation takes place. This is especially problematic for older patients relying on an RIC transplant, because most protocols will not allow >5% blasts. To be practical, HLA typing should be initiated immediately after the diagnosis of MDS. If a matched related transplant is not available, an unrelated donor search should be started while demethylating therapy is under way.

The lymphomas

Indolent lymphoma

Follicular lymphoma (FL) typically runs a relatively indolent course, but it is incurable with conventional chemotherapy. The addition of rituximab to frontline and salvage therapies has made a significant impact on extending the natural history of the disease. Thus, transplantation (either autologous or allogeneic) is reserved for salvage therapy. In the prerituximab area, the results of three large randomized trials from Europe suggested improved DFS but no benefit in OS for early remission patients randomized to the transplantation arm compared with conventional chemotherapy arm with

an incidence of therapy-related MDS ranging from 3.5% to 12% in the transplantation arms.

For patients with relapsed disease, only one 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 three cycles of salvage chemotherapy versus autologous SCT. After a median follow-up of 69 months, the hazard ratio for PFS favored the SCT arms compared with the salvage chemotherapy arms, and there was a trend for a superior OS favoring the high-dose therapy arms.

There appears to be a strong graft-versus-lymphoma effect in FL, and thus first ablative, and more recently, RIC approaches have been used in relapsed disease. Several non-randomized studies have shown a lower relapse rates after allografting compared with autologous transplant; but this gain was offset by the considerably higher cost of nonrelapse deaths with the ablative procedure. Recently RIC transplants have been used in FL, even in cases failing an autologous transplant. Two prospective studies have used fludarabine-based RIC conditioning regimens. The 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.

The incidence of transformation from FL to diffuse or Burkitt(-like) lymphoma is ~3% per year, with several studies reporting a risk of 30% by 10 years of follow-up. It is not known how the use of rituximab for FL will change (hopefully decrease) this rate. Chemotherapy alone is unlikely to be curative. Autologous transplant has been associated with a 5-year OS of ~40%-60%, with EFS or PFS ranging 25% to 50%, with the biggest barrier relapse. Ablative allogeneic transplants have not done better, because of high NRM. RIC approaches being studied, with PFS and OS ranging from 20% to >60%, likely owing to the differences in study populations (particularly chemoresponsiveness).

Aggressive NHL

Many patients with NHL can be cured with frontline combination chemotherapy or radiotherapy. For patients with suboptimal responses to initial therapy or for patients with relapsed or refractory disease, however, salvage therapy with chemotherapy alone typically is inadequate to achieve long-term survival. High-dose chemotherapy with autologous SCT offers curative potential and is the treatment of choice for patients with relapsed, chemotherapy-sensitive intermediate-grade lymphomas.

Autologous SCT is the standard of care for most patients with chemotherapy-sensitive relapsed or refractory-diffuse large-cell lymphoma. The international, multicenter, prospective PARMA trial was the pivotal study that established the role of autologous SCT for patients with relapsed, chemotherapy-sensitive, diffuse large B-cell NHL. In this trial, 109 of 215 patients who had relapsed diffuse large B-cell NHL and who responded to platinum-based salvage chemotherapy were assigned randomly to four more courses of conventional chemotherapy or autologous SCT. The 5-year EFS and OS were 46% and 53%, respectively, for the transplantation arm and 12% and 32%, respectively, for the chemotherapy arm. An important prognostic indicator was response to salvage chemotherapy. Patients with relapsed diffuse large B-cell NHL who were chemotherapy sensitive unequivocally fared better compared with patients with chemotherapy-resistant disease.

Patients with diffuse large B-cell NHL who demonstrate primary refractory disease or relapsed disease that is not responsive to salvage chemotherapy have poor outcomes even after autologous SCT. Autologous SCT 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 aggressive B-cell lymphoma, and rituximab has a growing role in the peritransplantation management of patients with aggressive lymphoma. Rituximab now commonly is added to salvage regimens (eg, R-ICE, R-DHAP) for added cytoreduction and because it also confers an *in vivo* purging effect and reduces the incidence of tumor contamination in the autograft. It also is given after transplantation as maintenance therapy to eradicate MRD in an effort to reduce recurrence.

Several prognostic factors are associated with outcome after autologous SCT. The International Prognostic Index (IPI) is the validated scoring system designed to predict survival of patients with newly diagnosed aggressive NHL. The IPI at relapse (second-line IPI), however, also has been shown to correlate with prognosis after autologous SCT. In addition, positron emission tomography (PET) scanning appears to have predictive value. Several studies have shown that PET positivity after salvage therapy is associated with an inferior failure-free survival (compared with PET negativity), independent of age-adjusted IPI. Other poor prognostic features include relapse within 12 months of diagnosis, advanced stage, poor performance status, and failure to achieve only a partial response (CR) after transplantation.

The use of autologous SCT as frontline therapy for aggressive lymphoma has been studied in phase 3 but has failed to show a benefit. Early autologous SCT confers higher CR rates but does not appear to improve EFS or OS compared

with conventional therapy in both good-risk and high-risk patients. The use of autologous SCT for patients with high-risk disease is being explored in clinical trials.

Allogeneic SCT is not offered routinely to patients with aggressive lymphoma. Exceptions include young patients with advanced disease, patients who failed to mobilize an adequate amount of PBSC, or patients who failed a previous autologous SCT. In a review of 101 patients with diffuse large B cell lymphoma (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 and chemo-refractory disease portended a worse outcome.

Mantle cell lymphoma

Mantle cell lymphoma is an unusual lymphoma (~5%-10% of lymphomas) and generally presents with advanced disease. Chemotherapy is poor in offering disease control, with a median OS of 3-5 years. Most younger newly diagnosed patients receive a high-dose cytarabine-containing regimen (eg, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone combined with rituximab) and obtain response rates of ~90% and failure-free survival rates of approximately 50%. Autologous SCT often is employed as consolidation therapy, although it is not clear whether autologous SCT significantly increases cure rates. For patients with relapsed or refractory disease, consideration of allogeneic SCT is preferred as it offers the only possible option for cure. Myeloablative allogeneic SCT can induce durable remissions in mantle cell lymphoma even in heavily pretreated patients, but it is associated with high upfront mortality. Therefore, reduced-intensity regimens increasingly are being offered, yielding EFS rates ranging from 50% to 85% even in patients who failed a prior autologous SCT.

As discussed in Chapter 18, children usually present with aggressive NHL and only rarely with low-grade lymphoma. Children with refractory disease or relapsed disease require SCT for long-term survival. Research is ongoing to determine whether there is a pediatric population at high risk for relapse who would warrant a transplant in first remission. Children and adults with HIV-associated NHL have undergone successful autologous SCT while on antiretroviral therapy.

Hodgkin lymphoma

For patients with relapsed or refractory disease, autologous SCT is the standard of care and confers cure rates of 40%-60% in patients with relapsed, chemotherapy-sensitive disease and 25%-40% in patients with chemotherapy-refractory disease. Before proceeding to autologous SCT, patients should receive salvage therapy for maximum cytoreduction,

with the platinum-based regimens being the most commonly used regimens.

The role of allogeneic SCT in patients with Hodgkin lymphoma is less established, and generally is pursued only in patients who have marrow involvement, have refractory disease, or have relapsed after an autologous SCT. In general, RIC transplant regimen is preferred to a fully ablative regimen, as RIC is associated with less regimen-related deaths and better survival. The Gruppo Italiano retrospectively compared nearly 200 Hodgkin cases following a failed autologous transplant, and divided the patients into those with a donor (sibling, unrelated, or haploidentical), versus those that could not secure a donor, with the intent that those with donor would have an RIC. The 2-year PFS and OS were superior in the donor group (39% vs. 14% and 66% vs. 42% retrospective). The Seattle group has compared the outcome for HLA-matched, unrelated matched, and haploidentical donors in RIC transplants and has found the survival to be similar in all approaches, with overall survival of ~60%, and PFS of ~40%. Chemosensitivity before the RIC is a strong determinant of subsequent relapse, and hence, PFS and overall survival.

The focus of first-line therapy for pediatric patients has been to decrease the toxicity of the current therapy while still achieving high cure rates. For children and adolescents who fail conventional chemotherapy, autologous SCT is offered as a curative option. The Children's Oncology Group is investigating the role of immune modulation to induce autologous GVHD in the immediate posttransplantation period. For those with bone marrow involvement or with relapse following an autologous transplantation, allogeneic transplantation has been performed and appears to be better tolerated than in the adult population.

Plasma cell dyscrasias

Multiple myeloma is the most common indication for autografting. When compared with chemotherapy, high-dose therapy with autologous SCT is associated with higher response rates and improved DFS and OS. When autologous SCT is given as part of the planned frontline treatment, ~22%-44% patients achieve CR, with median time to progression and OS time of 18-24 months and 4-6 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 SCT. The procedure is well tolerated, with an NRM of ~2%.

The advent of the immunomodulators, such as lenalidomide and thalidomide, and proteasome inhibitors, such as bortezomib, as 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 and provide the platform toward improving results with autologous SCT. Current guidelines state that high-dose chemotherapy with autologous SCT should be offered as initial 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 del(13), still have a poor outcome even after tandem (double) autologous SCT.

Because of concerns over the potential toxicities associated with SCT, a strategy of delayed transplantation has been tested. 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 QOL. Currently, the utility of planned tandem transplantation as the primary treatment remains controversial. In a randomized study from the French group, both response rates and survival favored tandem transplantation, in particular for patients with significant residual disease (the lack of at least a VGPR) after their first transplantations. The chance of event-free, relapse-free, and overall survival were 10%, 13%, and 21%, respectively, in the single-transplant group, compared with 20%, 23%, and 42% respectively in the double-transplant group. A recently published meta-analysis of six randomized trials with ~1,000 patients concluded that tandem autologous SCT confers higher response rates compared with single autologous SCT, but it did not find conclusive evidence for improvement in PFS or OS. A registry analysis from the EBMT group, however, demonstrated that when a second transplantation is performed within 3-6 months after the first transplantation, survival is improved.

Maintenance therapy after autologous SCT may decrease relapse rates. In two large randomized trials posttransplant lenalidomide therapy resulted in significant improvements in DFS. In the North American trial reported by McCarthy et al. (2012) lenalidomide maintenance therapy compared with placebo had less progression or death at study unblinding (20% vs. 44%, respectively), with a median time to progression of 46 months for lenalidomide versus 27 months for placebo. Lenalidomide had more predictable hematologic adverse events, however, and more secondary primary cancers occurred in lenalidomide patients (8%) compare with placebo (3%).

Relapse is the overwhelming cause of autologous SCT failure, either owing to tumor contamination of PBSC or lack of a graft-versus-myeloma effect. Allografting addresses both issues, but ablative allografts have been associated with a very high NRM. Thus, there has been interest in using

RIC transplants after an autologous transplant. CR rates are >50%, with 5-year overall survival ~60%, and PFS/EFS 30%-50%. Relapse is still a problem even after the allograft, as approximately 7% per year of cases continue to relapse. Like other modalities, the del(13q) karyotype is associated with an inferior outcome. A recently conducted trial of biologic assignment to allogeneic SCT performed by the BMT-CTN failed to show a benefit for patients undergoing an allogeneic SCT as consolidation of an autograft.

AL (light-chain amyloidosis) amyloidosis is a clonal plasma cell disorder similar to multiple myeloma and characterized by widespread disposition of amorphous extracellular materials 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 ~1-2 years. Although conventional chemotherapy has limited utility in patients with AL, autologous SCT can reverse the disease process for selected patients with AL. Nonimmunoglobulin forms of amyloidosis will not benefit from cytotoxic therapy, including transplantation. Because of the preexisting organ dysfunction in patients with AL amyloidosis, despite careful patient selection, the NRM is still 4-8 times (thus ~25%) higher compared with that of autologous transplantation for multiple myeloma (~5%). The causes of NRM include gastrointestinal bleeding, cardiac arrhythmias, and the development of intractable hypotension and multiorgan failure. Several studies have suggested a 2-3 year survival of ~70%, following autologous transplant, although patients with multiorgan involvement have a distinctly worse survival. A phase III trial in which AL amyloid patients were randomized to receive autologous SCT versus 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 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. Because POEMS syndrome is a rare disease, it would be difficult to perform any prospective study to define the exact role of SCT. Nevertheless, SCT results in significant clinical improvement in a majority of patients and should be considered a therapeutic option in these patients.

Aplastic anemia and other autoimmune diseases

The therapy of aplastic anemia depends on the severity of the aplasia, the availability of a matched related donor, and the age of the patient. The standard first-line therapy for a newly diagnosed patient with severe aplastic anemia is allogeneic transplantation if a matched related donor is available; if not, immunosuppressive therapy (IST) with some combinations of cyclosporine and ATG, with unrelated transplant reserved for patients who do not adequately respond to IST. Long-term survival following a matched related transplantation exceeds 80%. Inferior survival is associated with older age and use of an unrelated donor. The main complication of transplant is related to chronic GVHD, thus bone marrow rather than peripheral blood is the highly preferred source of stem cells. In regards to preparative regimen, most use high-dose Cytoxan (50 mg/kg \times 4 doses) with ATG.

For young patients with newly diagnosed idiopathic severe aplastic anemia and an HLA-identical sibling, many centers recommend immediate transplantation to minimize the sensitization with transfusions, which historically has resulted in an increased risk for graft rejection. Although the use of cyclosporine, as well as the use of leukodepleted blood products, has reduced the problem of rejection, sensitization should be minimized; directed family donations of blood products should be avoided. Indeed, one large study showed the hazard ratio for mortality was 1.7 for patients who received IST before transplant, compared with those patients who underwent front-line transplantation. Secondary malignancies are not uncommon after transplantation for aplastic anemia and may occur in as many of 10% of cases 15 years from transplant. Risk factors include age >15 years, use of cyclosporine (CSP) 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 rule out Fanconi anemia in potential transplantation recipients. 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 reduced-intensity 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. Much like for other inherited disorders, siblings should be screened for HLA compatibility and to rule out the presence of the disease.

There has been increasing interest in autologous transplantation as a method of treating life-threatening autoimmune disorders. Autologous transplantations have been used in

multiple sclerosis, systemic sclerosis (usually for lung disease), rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus, dermatomyositis/polymyositis, Crohn's 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 will depend on the results of ongoing trials, some general observations are now possible. First, allogeneic SCT (ASCT) has considerable treatment-related morbidity and even mortality. This has been particularly true in patients with advanced multiple sclerosis. Second, the underlying organ dysfunction often progresses acutely through the transplantation, even if there is stability to improvement later. Third, durability of response and the need for continued therapy 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 team has pioneered transplantation for thalassemia and has 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 one or two factors, and class III patients have all three 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 transplantations 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. Results from the 150 children who have received transplantation from HLA-identical siblings have a >90% survival rate and 85% are disease free. Moreover, successful SCT appears to prevent further sickle cell complications. A study from Belgium demonstrated that patients who received transplantation early in the course of their disease (less than four blood transfusions) had a 100% survival rate and 93% DFS rate compared with an 88% survival 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.

Nonmyeloablative allografting has been studied in adults. Recently, a new preparative regimen, including pretransplant alemtuzumab (an antibody therapy to CD52, which reduces host B- and T-cells), 300 cGy of TBI, and posttransplant sirilimus 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 were stable-donor chimerism. Remarkable, 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 important to predict the course of the disease and to be able to tailor therapy appropriately. The most common diseases for which 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 SCT. 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 SCT is established. SCT approaches are modified based on the exact diagnosis. The need for a preparative regimen and its intensity are determined in part by the function of the lymphocytes and NK cells.

SCT 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 HLA molecules. 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 stem cells. In these patients, simple infusion of stem cells is usually all that is required, without a preceding preparative regimen. Many of the recipients who received stem cells without a preparative regimen failed to develop normal B-cell function and required ongoing IgG replacement. 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 transplantations, 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 have been corrected with SCT. 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 are not treatable by transplantation. Other disorders such as globoid cell leukodystrophy; metachromatic leukodystrophy; adrenoleukodystrophy; mannosidosis; fucosidosis; aspartyl-glucosaminuria; Hurler, Hunter, Maroteaux-Lamy, and Sly syndromes; and Gaucher disease type III have been treated successfully with SCT. 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 will 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 matched unrelated donor unrealistic, thus making cord stem cells more attractive in these cases. GVHD in some of these disorders (eg, adrenal leukodystrophy) 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 adrenal leukodystrophy, 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, SCT will halt the disease progression, but the patient may not regain lost milestones or function.

SCT for solid tumors

Germ cell cancer

Germ cell cancer is highly curable, even in patients with disseminated disease. Although conventional-dose cisplatin-based chemotherapy cures the majority of patients, 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 failed salvage chemotherapy or had cisplatin-refractory disease ultimately died of the disease. Approximately 15%-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 SCT. 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 group prospective study, patients were randomized between four cycles of etoposide, ifosfamide, and cisplatin (VIP) versus three cycles of VIP plus a single cycle of high-dose therapy followed by ASCT. The 3-year EFS for patients who received VIP only was 35% versus 42% for patients randomized to transplantation, with no difference in OS. A U.S. intergroup phase III 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 SCT 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 SCT 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 EFS was superior for the SCT group (34% vs. 22% at 3 years). Among patients assigned to receive *cis*-retinoic acid and SCT, EFS was 55% versus 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 SCT-related mortality. Ongoing studies are investigating additional SCT-related strategies to further improve the outcome of high-risk patients, such as the use of sequential autologous transplantations (tandem transplantations), combination therapies with high-dose radiopharmaceutical agents such as iodine-131 meta-iodobenzylguanidine, and ASCT.

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 0.8 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; several large retrospective studies have failed to show a clear benefit from SCT compared with conventional therapies. The prognosis still remains poor for those receiving transplantation in the setting of residual disease. In a study from the National Cancer Institute, 91 patients were enrolled on a series of three protocols consisting of induction chemotherapy, radiation to the primary site, consolidation with TBI (8 Gy), and autologous BMT. 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 SCT.

Summary

SCT is a rapidly evolving field. Results have improved over the past decade, and indications have changed. Transplantation is more widely applicable because of improvements in supportive care and donor selection and the advent of RIC regimens. For patients with malignant diseases, especially the lymphoid malignancies, the chance for a better outcome is significantly improved if patients are referred 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 time for SCT.

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Myeloid disorders and congenital marrow failure syndromes
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CHAPTER
15



Myeloid disorders and congenital marrow failure syndromes

Geoffrey L. Uy and Inderjeet Dokal

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The term *myeloid* derives from the Greek *myelos*, meaning “marrow,” and is used to describe hematologic conditions or diseases not involving the lymphoid tissues or lymphocytes. “Myeloid” is also used to describe disorders primarily involving granulocytes (neutrophils, eosinophils, or basophils) and monocytes. Tissue macrophages (histiocytes), Langerhans cells, and interdigitating dendritic cells also arise from monocytic progenitors or precursors. Other important immunoregulatory dendritic cell types and mast cells derive from marrow progenitors that are distinct from myeloid and monocytic progenitors.

Bone marrow (BM) failure refers to the inability of hematopoiesis to meet physiologic demands for production of healthy blood cells. Pancytopenia may result from marrow failure, or cytopenias involving a single myeloid lineage may dominate; usually lymphopoiesis is relatively preserved. BM failure syndromes can be classified into idiopathic, congenital or inherited, and secondary. The range of molecular mechanisms responsible for congenital marrow failure states (discussed in this chapter) is broad, including abnormal DNA-damage response (Fanconi anemia [FA]), defective ribosome biogenesis (Diamond-Blackfan anemia [DBA]), abnormal telomere dynamics (dyskeratosis congenita), and

altered hematopoietic growth factor receptor–kinase signaling (congenital amegakaryocytic thrombocytopenia). In some congenital marrow failure syndromes, the mechanism of hematopoietic failure is currently unclear.

Granulocytes: neutrophils, eosinophils, and basophils

The term *granulocytes* refers to circulating neutrophils, eosinophils, and basophils, although because of their predominance in the blood, the terms *neutrophil* and *granulocyte* sometimes are used synonymously. Normal values for the differential count for leukocytes in the blood are shown in Table 15-1. Neutrophils are a critical component of the innate immune response, and persistent neutropenia is associated with a marked susceptibility to infection. Evidence also is increasing that neutrophils are a major contributor to tissue damage in inflammatory diseases. Neutrophil homeostasis in the blood is regulated at three levels: neutrophil production in the BM (granulopoiesis), neutrophil release from the BM to blood, and neutrophil clearance from the blood (Figure 15-1).

Granulopoiesis

Under normal conditions, neutrophils are produced exclusively in the BM, where it is estimated that 10^{12} are generated on a daily basis. Granulocytic differentiation of hematopoietic stem cells (HSCs) is regulated by the coordinated expression of a number of key myeloid transcription factors, including PU.1, CCAAT enhancer-binding protein α (C/EBPα), C/EBPε, and GFI-1. A number of hematopoietic growth factors provide extrinsic signals that regulate granulopoiesis. The

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Table 15-1 Normal leukocyte values in peripheral blood in adults.

	CONVENTIONAL UNITS	SI UNITS
Leukocyte counts	Non-blacks: 4,000-10,000/mm ³ Blacks: 2,800-10,000/mm ³	4.0-10 × 10 ⁹ /L 2.8-10 × 10 ⁹ /L
Leukocyte differential	(% of total)	(Absolute K/mm ³)
Leukocyte differential	(% of total)	(Absolute K/mm ³)
Neutrophils	40-70	1.4-7.5
Bands	0-15	0-1.5
Lymphocytes	20-50	0.8-5.0
Monocytes	3-8	0.1-1.0
Eosinophils	0-4	0-0.4

most important of these is granulocyte colony-stimulating factor (G-CSF), which stimulates the proliferation of granulocytic precursors, reduces the average transit time through the granulocytic compartment, and stimulates neutrophil release from the BM.

Neutrophil release

Neutrophils are released from the marrow 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. A broad range of substances has been shown to induce neutrophil release from the BM, including chemokines, cytokines, microbial products, and various other inflammatory mediators (eg, C5a). Recent evidence suggests that the chemokine stromal derived factor-1 (SDF1, CXCL12) plays a key role in regulating neutrophil trafficking in the BM.

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 are cleared rapidly with a half-life of only 6-8 hours. Neutrophils are cleared primarily in the liver, spleen, or BM, where apoptotic or aged neutrophils are phagocytosed by macrophages.

Neutrophil extravasation

Neutrophils in the circulation loosely attach and subsequently adhere to vascular endothelium in response to the local production of inflammatory cytokines and chemokines (Figure 15-1). 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, a rare syndrome

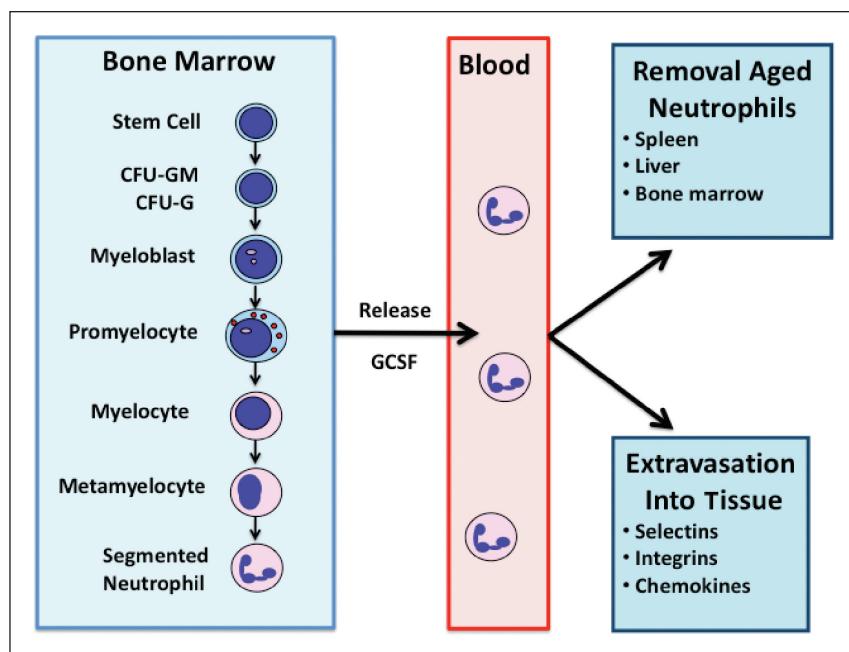


Figure 15-1 Neutrophil homeostasis. The bone marrow is the primary site of granulopoiesis in humans. Under basal conditions, the bone marrow contains a large reservoir of mature neutrophil. Neutrophils are released into the circulation in a regulated fashion. The principal cytokine regulating both granulopoiesis and neutrophil release into the blood is granulocyte colony-stimulating factor (G-CSF). Neutrophils in the circulation have two general fates. In response to local infection or inflammation, neutrophils can emigrate into tissue. Neutrophil emigration is a highly orchestrated process that includes sensing of chemokine gradients, selectin-mediated rolling on inflamed endothelium, and integrin-mediated adhesion and diapedesis through the endothelium. Alternatively, senescent (aged) neutrophils are cleared from the circulation primarily by the macrophages in the spleen, liver, and bone marrow.

manifested by normal neutrophil production but impaired emigration to sites of inflammation.

Once emigrated to the inflammatory site, neutrophils function primarily as tissue phagocytes. These vital functions depend on several critical features of these cells. 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 glycogen are involved in the intracellular oxidative burst that accompanies phagocytosis and in the killing and digesting of microorganisms. Maintenance of both the supply of cells and the integrity of all of these functions is critical for normal host defense mechanisms. Diseases affecting each of these features and functions of neutrophils result in enhanced susceptibility to infection.

Eosinophils and basophils

Normally, the marrow contains a small proportion of eosinophils and basophils. The granules of eosinophils contain histamine and proteins important for the killing of parasites. Eosinophil production is increased and eosinophilia (ie, $>0.7 \times 10^9/L$) occurs in allergic disorders (ie, asthma, allergic rhinitis, dermatitis), parasitic infections, collagen vascular dis-

eases, and drug reactions. Eosinophilia also frequently occurs with myeloproliferative disorders and is the hallmark feature of the hypereosinophilic syndrome, a disorder characterized by autonomous eosinophil production with end-organ or tissue complications.

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 the immediate (type 1) hypersensitivity responses. Increases in basophils are associated with hypersensitivity reactions, including drug and food allergies. Basophilia is a common feature of myeloproliferative disorders, particularly chronic myelogenous leukemia. It also can be associated with other chronic inflammatory diseases, such as tuberculosis, ulcerative colitis, and rheumatoid arthritis, but it rarely is seen as an isolated finding.

Neutrophilia

Neutrophilia is an excess of circulating neutrophils and is typically defined as an *absolute neutrophil count* (ANC) >2 standard deviations above the mean. Neutrophilia is associated with many different types of stress, including recent exercise, infection, and inflammatory diseases (Table 15-2).

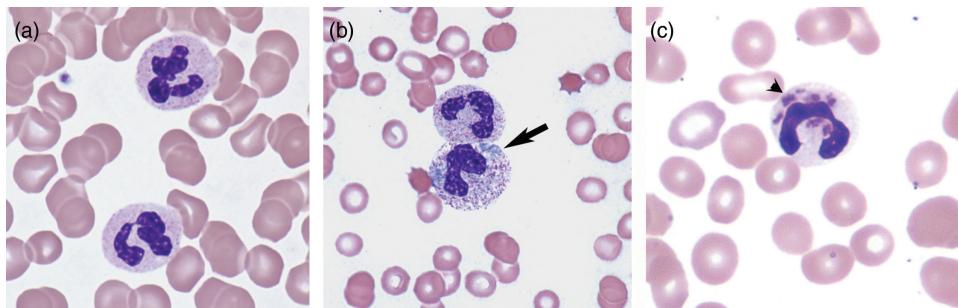
Table 15-2 Causes of neutrophilia.

Acute neutrophilia	Chronic neutrophilia
<i>Acute infections</i> Many localized and systemic acute bacterial, mycotic, rickettsial, spirochetal, and certain viral infections	<i>Chronic Infections</i>
<i>Inflammation or tissue necrosis</i> Burns, electric shock, trauma, myocardial infarction, gout, vasculitis, antigen–antibody complexes, complement activation	<i>Inflammation</i> 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
<i>Physical or emotional stimuli</i> Cold, heat, exercise, convulsions, pain, labor, anesthesia, surgery, severe stress	<i>Tumors</i> Gastric, bronchogenic, breast, renal, hepatic, pancreatic, uterine, and squamous cell cancers
<i>Drugs, hormones, and toxins</i> Epinephrine, etiocholanolone, endotoxin, glucocorticoids, venoms, vaccines, colony-stimulating factors	<i>Drugs, hormones, and toxins</i> Cigarette smoking, continued exposure to many substances that produce acute neutrophilia; lithium; rarely, as a reaction to other drugs
	<i>Metabolic and endocrinologic disorders</i> Pregnancy and lactation, eclampsia, thyroid storm, Cushing disease
	<i>Hematologic disorders</i> Rebound from agranulocytosis or therapy of megaloblastic anemia, chronic hemolysis or hemorrhage, asplenia, myeloproliferative disorders
	<i>Heredity and congenital disorders</i> Down syndrome, familial Mediterranean fever, leukocyte adhesion deficiency, hereditary neutrophilia
	<i>Chronic idiopathic neutrophilia</i>

A prompt increase in the blood neutrophil count, as well as the circulating levels of other leukocytes, occurs with acute stress, exercise, anxiety, and epinephrine administration. Only rarely does this response more than double the count. It is attributable to demargination of cells, not to the release of cells from the marrow reserve. Normally, approximately one-half of the neutrophils in the circulation are loosely adherent to blood vessels and not counted in a routine blood count. These cells are described as being in the marginal pool. The other half of the neutrophils are circulating freely with the red cells and platelets. They are described as being in the circulating pool.

Neutrophilia associated with infections and inflammatory disorders occurs by two 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 response associated with infections may stimulate 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, mostly including band forms as well as metamyelocytes and occasionally early granulocytic precursors. In addition, there often is a change in the morphology of neutrophils with the appearance of vacuoles and “toxic granulation,” because of more intense staining of the primary granules of neutrophils. Cells released prematurely also may contain bits of endoplasmic reticulum that stain as blue bodies in the cytoplasm, called *Döhle* bodies (Figure 15-2).

Neutrophilia is also a feature of the myeloproliferative syndromes, particularly chronic myelogenous leukemia (CML) and chronic myelomonocytic leukemia (CMML). In most cases of reactive neutrophilia, the inciting infection (or other stress) is usually clinically obvious, and the neutrophilia is self-limited. In patients without demonstration 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.



In rare cases, neutrophilia may be due to intrinsic defects of neutrophil function. Leukocyte adhesion deficiency and familial Mediterranean fever are associated with neutrophilia; they are discussed in detail in the section on disorders of neutrophil function.

Neutropenia

Neutropenia is defined as an ANC of <1,500 cells/µL. Neutrophil levels are lower in some ethnic and racial groups (eg, Africans, African Americans, and Yemenite Jews). For example, in adult African Americans, the 95% confidence limits for the ANC are 1,300-7,400 cells/µL. A survey of 25,222 participants in the National Health and Nutrition Examination Survey showed that 4.5% of black participants had an ANC of <1,500/µL, compared with 0.79% and 0.35% of whites and Mexican Americans, respectively.

Neutropenia is classified based on the ANC as severe (<500/µL), moderate (500-1,000/µL), or mild (1,000-1,500/µL). The risk of infection begins to increase with an ANC <1,000/µL. 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 less common, and there is no increase in susceptibility to viral or parasitic infections. The differential diagnosis of neutropenia is broad (Table 15-3). Chemotherapy-induced neutropenia is a common complication of the treatment of cancer after treatment with myelotoxic drugs. Neutropenia is a frequent manifestation of myelodysplastic syndromes (MDS), acute leukemia, and marrow-infiltrative processes, such as myelofibrosis, or metastatic carcinoma; they are discussed in detail in their respective chapters.

Monocytes and tissue histiocytes

Monocytes share their origin and functions with neutrophils. They are phagocytes, ingesting and killing microorganisms. Monocytes also may become the fixed phagocytic cells that line portions of the circulation, particularly in the spleen and liver. In these tissues, their role is to clear particulate matter, including microorganisms, and aged or damaged

Figure 15-2 Photomicrographs of blood smears showing typical neutrophil morphology from (a) a healthy individual; (b) a patient with sepsis showing Döhle bodies (arrows) and toxic granulations; (c) a patient with Chédiak-Higashi syndrome showing the large cytoplasmic inclusions (arrowhead). From ASH Image Bank, #3780-3378-2979.

Table 15-3 Causes of neutropenia.

I. Congenital neutropenia syndromes
Severe congenital neutropenia (Kostmann syndrome)
Cyclic neutropenia
Shwachman-Diamond syndrome
WHIM syndrome (Myelokathexis)
Disorders of vesicular transport
Chédiak-Higashi syndrome
Griscelli syndrome, type II
Hermansky-Pudlak syndrome, type II
p14 deficiency
Cohen syndrome
Barth syndrome
Cartilage-hair hypoplasia syndrome
Pearson's syndrome
Glycogen storage disease type 1b
Dyskeratosis congenita
Neutropenia associated with immunodeficiency syndromes
II. Acquired neutropenia
Neonatal alloimmune neutropenia
Primary autoimmune neutropenia
Secondary autoimmune neutropenia
Systemic lupus erythematosus
Felty syndrome
Nutritional deficiencies
Vitamin B12, folic acid, copper
Myelodysplastic syndrome
Acute leukemia
Myelophthysis (bone marrow infiltration tumor, fibrosis, granulomas)
Large granular lymphocytic leukemia
Neutropenia associated with infectious disease
Sepsis
Rickettsial: human granulocytic ehrlichiosis
Viral: mononucleosis, HIV
Drug-induced neutropenia
Hypersplenism

blood cells from the circulation. They are also an important source of inflammatory cytokines (eg, tumor necrosis factor, interleukin-1, interferon- γ) that cause fever and many of the symptoms associated with infectious and inflammatory diseases. Monocytes can differentiate to tissue histiocytes, and these cells may then fuse to form giant cells, as occurs in tuberculosis and sarcoidosis. Alveolar macrophages in the lung, Kupffer cells in the liver, and osteoclasts and phagocytic cells in reticuloendothelial tissue are all forms of tissue histiocytes that are thought to derive from blood monocytes or, in some cases, from circulating monocyte progenitors. These phagocytic cells not only serve antimicrobial, scavenger, and secretory functions, but also participate in wound repair and antigen processing and presentation. Chronic inflammatory

states may increase monocytes in the blood and the tissues. Transient monocytopenia occurs with stress and infections. In aplastic anemia (AA) and hairy cell leukemia, monocyte numbers are suppressed, but circulating monocyte counts and function are maintained in many other conditions that cause neutropenia. Inappropriate overstimulation of tissue macrophages, usually in the setting of infectious stimuli with or without underlying acquired or congenital immune dysregulation, may contribute to the sepsis-associated systemic inflammatory response syndrome.

Dendritic cells

Dendritic cells (DCs) are hematopoietic-derived cells that participate in the innate and adaptive immune responses. These cells are distributed widely throughout virtually all tissue types and, in particular, lymphoid organs and organs with barrier function, such as the skin and mucosal surfaces. A major function of DCs is to process and present antigen to the immune system. Immature DCs in the peripheral tissue express surface receptors that allow them to recognize and take up extracellular antigens in their environment. Upon encountering antigen, immature DCs undergo activation and maturation and migrate to secondary lymphoid organs where they bind and present antigen in the context of major histocompatibility complex (MHC) class I and II molecules to stimulate naive T-cells. Mature DCs also produce cytokines, which can prime T-cells toward either a T_H1 versus T_H2 response. In addition, DCs possess other immunomodulatory functions, including the activation of other lymphocyte subsets and induction of immune tolerance. Specific DC subsets have been identified and characterized based on their location, cell surface phenotype, function, or developmental stage. The ability of DCs to present tumor antigens has led to their use in vaccine immunotherapy trials. An important pathologic condition of DCs is Langerhans cell histiocytosis, an acquired clonal disorder that can lead to local or systemic tissue infiltration and organ failure.

Moncytosis

Monocytes normally account for approximately 1%-9% of peripheral blood leukocytes, with absolute monocyte counts ranging from $0.3\text{-}0.7 \times 10^9/\text{L}$. An increase in circulating monocytes may be observed in chronic inflammatory conditions and infectious diseases, such as tuberculosis, endocarditis, and syphilis. Moncytosis is a hallmark of chronic and juvenile myelomonocytic leukemias and also may be observed in other malignancies, including lymphomas and acute monocytic leukemias. In the inflammatory conditions, moncytosis is a reactive process resulting from the peripheral production of cytokines, which stimulate monocyte production. Malignant moncytosis is presumed to be due

to specific molecular defects affecting monocyte proliferation, differentiation, and survival.

Moncytopenia

A decreased absolute monocyte count can be encountered in BM failure states such as AA and, less commonly, MDS. Moncytopenia, along with neutropenia, is characteristic of hairy cell leukemia. Low monocyte counts are encountered with overwhelming sepsis and as the result of cytotoxic chemotherapy.

Congenital marrow failure syndromes

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 spicules 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

Although the inherited BM failure syndromes are rare disorders, collectively affecting just a few dozen new patients in the United States each year, a diagnosis with one of these syndromes has profound implications for medical management and treatment. FA is probably the most common and one of the best defined of these rare congenital conditions (common is a relative term here, as the incidence of FA in the United States has been estimated at approximately 1 in 360,000 live births).

Several inherited marrow failure syndromes are compared in Table 15-4. BM failure usually is not the only feature of the congenital marrow failure disorders, and marrow failure may even be absent in some patients. Alternatively, isolated marrow failure or development of a malignancy may be the first clinical manifestation of FA in the absence of other clinical stigmata, and occasionally, patients with FA may first come to clinical attention as young adults.

Pathophysiology

A hallmark of cells from patients with FA is hypersensitivity to genetic damage induced by DNA-damaging and cross-linking agents, such as DEB and mitomycin C (MMC). The underlying molecular defects resulting in the FA phenotype involve components of a critical pathway that regulates cellular DNA-damage recognition and response.

FA is a heterogeneous disease at the molecular level, with at least 15 distinct complementation groups (ie, distinct genes; *complementation* in molecular biology means that two different gene loci encode proteins of distinct function that can each provide something the other lacks, facilitating identification *in vitro*) identified to date. More than 75% of patients fall into complementation groups A or C. Molecular cloning of the genes corresponding to each of these complementation groups led to the identification of a complex DNA repair pathway composed of distinct FA proteins (Figure 15-3). Each of these genes, when biallelically mutated, can cause FA.

DNA-damage activates an eight-protein core complex consisting of Fanconi A, B, C, E, F, G, L, and M proteins, which results in monoubiquitylation of the Fanconi I (FANCI) protein and the Fanconi D2 (FANCD2) protein, probably by a ubiquitin ligase domain of FANCL. (Confusingly, there is no Fanconi H protein; it turned out to be the same complementation group as Fanconi A. There is also no Fanconi K protein.) The modified FANCD2 protein then translocates to chromatin nuclear foci and localizes to sites of DNA repair, where it interacts with the breast cancer susceptibility protein BRCA1, as well as with effector proteins FANCJ (a structure-specific helicase that binds to BRCA1) and FANCN. FANCD2 is phosphorylated by the ataxia-telangiectasia-mutated (ATM) protein kinase in response to ionizing radiation, linking the Fanconi pathway to the ATM/ATR/Chk1 DNA damage-sensing and checkpoint response pathway.

Biallelic mutations in the *BRCA2* gene, encoding a DNA repair enzyme for which heterozygous mutations had been linked to breast, ovarian, and prostate cancer susceptibility, were shown to be the underlying abnormality in FA patients who previously were assigned to complementation group D1; thus, *BRCA2* and *FANCD1* turned out to be identical. *FANCD1/BRCA2* is essential for recruiting the homologous recombination-promoting protein RAD51 into DNA-damage-inducible nuclear foci. These data clearly establish a defect in the ability to repair certain types of DNA damage appropriately as the underlying abnormality in FA, although the precise molecular mechanisms are still being elucidated.

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

Table 15-4 Comparison of the congenital marrow failure syndromes.

Syndrome	Male: female ratio	Percent diagnosed >15 years	Somatic features	Hematological features	Nonhematological cancers	Screening test	Genetics (genes)
Fanconi anemia (FA)	1.2:1	6.6	9	Skin hyperpigmentation and café-au-lait spots, short stature, triangular face, abnormal thumbs/radii, microcephaly; abnormal kidneys, decreased fertility	Pancytopenia, hypocellular bone marrow, MDS, leukemia	Solid tumors (head, neck, gynecologic, liver, CNS)	Autosomal recessive (14), X-linked recessive (1)
Dyskeratosis congenita (DC)	4:1	15	46	Nail dystrophy, abnormal skin pigmentation, leukoplakia, lacrimal duct stenosis, pulmonary fibrosis, liver fibrosis, esophageal strictures, early gray hair, osteoporosis, cerebellar hypoplasia, retinopathy, hypogonadism, urethral stricture	Pancytopenia, hypocellular marrow, MDS, leukemia	Solid tumors (head and neck)	X-linked recessive (1), autosomal dominant (3), autosomal recessive (6)
Diamond-Blackfan anemia (DBA)	1.1:1	0.25	1	Short stature, abnormal thumbs, hypertelorism, cardiac septal defect, cleft lip or palate, short neck	Macrocytic anemia, erythroid hypoplasia in marrow, MDS, leukemia	Solid tumors (osteosarcoma)	Autosomal dominant (10)
Shwachman-Diamond syndrome (SDS)	1.5:1	1	5	Short stature, exocrine pancreatic insufficiency with malabsorption	Neutropenia, anemia, thrombocytopenia, aplastic anemia, MDS, leukemia	None	Autosomal recessive (1)
Severe congenital neutropenia (SCN)	1.2:1	3	13	None	Neutropenia, MDS, leukemia	None	Autosomal dominant and recessive (5)
Congenital amegakaryocytic thrombocytopenia (CAMT)	0.8:1	0.1	0	Usually none	Thrombocytopenia; decreased megakaryocytes initially; later aplastic anemia; MDS, leukemia	None	Autosomal recessive (1)
Thrombocytopenia absent radii syndrome (TAR)	0.7:1	0.6	0	Absent radii, abnormal ulnae or humeri (phocomelia), thumbs present, occasional cryptorchidism, hypertelorism, horseshoe kidney, hemangiomas, macroglossia, cow's milk allergy, cardiac anomalies	Thrombocytopenia, MDS, leukemia	Bone marrow exam for megakaryocytes	Autosomal recessive (1)

CNS = central nervous system; DEB = diepoxybutane; MDS = myelodysplasia; MMC = mitomycin-C.

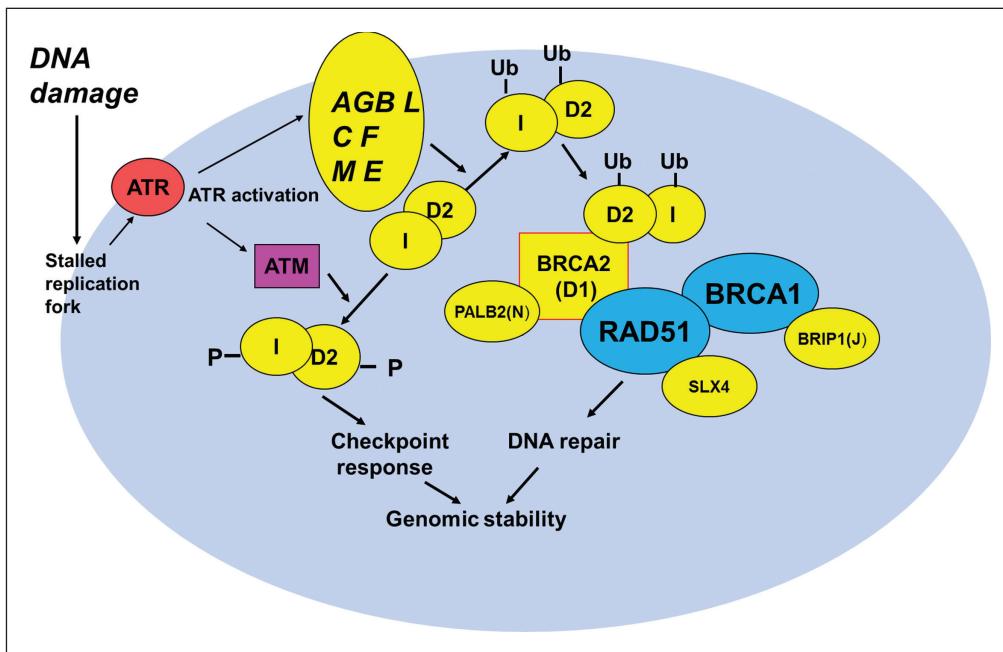


Figure 15-3 A model of the Fanconi anemia (FA) pathway. The FA core complex consists of eight FA proteins (A, B, C, E, F, G, L, and M) and this together with ATR (Ataxia-Telangiectasia and RAD3 related protein) is essential for the ubiquitination-activation of I-D2 complex after DNA damage. Activated 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.

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 FA patients.

Cells from patients with FA have been shown to have increased sensitivity to apoptosis, and elevated levels of apoptosis may contribute to the hematopoietic failure phenotype. Additionally, FA hematopoietic progenitor cells are hypersensitive to interferon- α , a known inhibitor of hematopoiesis.

Clinical features and diagnosis of FA

FA is an autosomal-recessive (usually) or X-linked recessive (rarely) disorder that is characterized by pancytopenia and congenital defects in the cutaneous, musculoskeletal, and urogenital systems. Patients with FA were recognized historically by characteristic physical findings, including short stature, microcephaly, intense patchy brown pigmentation of the skin, gonadal abnormalities, and malformations of the thumb and kidney. Hemoglobin F levels are increased in FA, and 80% of patients develop signs of BM failure by age 20 years. It is now clear that many patients (approximately 30%) with FA lack any abnormal physical findings, and some may not demonstrate overt marrow failure, so a high index of

suspicion is required. Chromosome breakage testing now secures the diagnosis in most patients.

Contemporary diagnosis of FA is based on the analysis for chromosome breaks in phytohemagglutinin-stimulated peripheral blood lymphocytes cultured with and without clastogenic agents (DEB or MMC). Results usually are reported as percentage of cells with chromosome aberrations; the percentage of such cells inducible in samples from healthy individuals depends on the specific laboratory protocol but is increased dramatically in FA. Alternatively, the finding of an increased percentage of cells arrested at the G2/M phase (4 N DNA) of the cell cycle, ascertained by flow cytometry, also is consistent with a diagnosis of FA. BM cells should not be used for chromosome breakage studies because false-negative results are more likely. These in vitro diagnostic tests have revealed that ~30% of patients with FA lack typical physical findings, and ~10% of patients first present at age >16 years.

Diagnosis may be complicated by the development of somatic mosaicism in the lymphocytes. Somatic mosaicism results from a genetic reversion to normal (non-FA), such that a subset of lymphocytes is no longer susceptible to 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.

Although spontaneous chromosomal alterations can be observed in Bloom syndrome and ataxia telangiectasia, DEB-induced chromosomal alterations are not seen in those disorders. Patients with Nijmegen breakage syndrome (NBS) may exhibit increased chromosomal breakage with MMC, and this condition must be distinguished from FA; immunologic abnormalities are characteristic of NBS but not typically in FA, and in doubtful cases, testing of the *NBS1* gene can help diagnostically.

Complications of marrow failure are the most common causes of death in FA, but FA also is characterized by an increased incidence of malignancies. Approximately 10%-15% of FA patients develop MDS or AML, often in the context of a hypoplastic marrow and monosomy 7. Patients with FA also are at increased risk for liver tumors, and squamous cell carcinomas (particularly esophageal, oral, and vulva/vaginal tumors) are much more common in patients with FA than in the general population. In addition to hepatocellular carcinoma, peliosis hepatitis and hepatic adenomas also 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; 30% of patients with FA will develop a solid tumor by age 45 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 FA patients 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 marrow failure in FA patients is allogeneic HSC transplantation (HSCT). Because of the increased sensitivity of FA cells to DNA damage-inducing agents, it is necessary to use modified transplantation conditioning regimens, such as attenuated alkylating agent and radiation doses, or alternative non-DNA-damaging agents, such as fludarabine or antithymocyte globulin (ATG). For this reason, it is critical to identify patients with FA as having the condition before proceeding to stem cell transplantation. Patients with FA who present with MDS or AML without an observed BM failure phase may go

unrecognized until the use of standard transplantation conditioning results in a disaster.

Stem cell transplantation in FA is associated with an increased risk of subsequent solid tumors, particularly in the setting of chronic graft-versus-host disease (GVHD). Stem cell transplantation corrects only the hematopoietic defect, and the patient remains at risk for FA-related complications in other tissues, such as solid tumors. Despite these limitations, stem cell transplantation from a matched (unaffected) sibling may be considered as the initial treatment of choice for patients with FA who present with marrow failure. Unrelated donor stem cell transplantation is complicated in these patients because the intensive conditioning regimens required for engraftment can exacerbate cellular damage and increase the risk for GVHD. 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/d) may elevate the blood counts in a subset of patients. Although the response to androgen therapy typically is most pronounced in the red blood cell lineage, improvements in platelet and neutrophil counts also have been seen. 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 or granulocyte-macrophage colony-stimulating factor (GM-CSF). Some patients who initially respond to androgens may become refractory over time. Supportive therapy with transfusions can be considered, but in the patient who is a candidate for allogeneic stem cell transplantation, 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 defect in DNA repair.
- Approximately 80% of patients develop signs of marrow failure, but the absence of marrow failure does not rule out FA if typical physical stigmata are present.
- The diagnostic test for FA is a DEB or MMC chromosome breakage study.

Key points (continued)

- FA can present in adulthood and without classic features other than BM failure. These patients remain at risk for marrow failure, leukemia, and solid tumors.
- Chemotherapeutic agents and radiation are poorly tolerated; attenuated conditioning regimens are necessary for stem cell transplantation.
- Stem cell transplantation is the only curative option for FA-associated hematologic manifestations.
- Careful monitoring for malignancies allows early institution of treatment, with attention to minimizing exposure to chemotherapy and radiation.

Dyskeratosis congenita

Clinical case

A 16-year-old boy presents to his doctor with a history of skin rash, nail abnormalities, and bruising. Following referral to the hematologist, examination showed he has significant nail dystrophy and reticulate skin pigmentation around the neck. Blood count reveals a nonsevere pancytopenia and the BM cellularity is found to be markedly reduced. Peripheral blood chromosomal breakage analysis following exposure to DEB was normal. Subsequent tests, however, showed he has very short telomeres and a missense mutation in the *DKC1* gene, confirming a diagnosis of X-linked dyskeratosis congenita.

Clinical features

The spectrum of diseases encompassed by the term dyskeratosis congenita (DC) has expanded considerably since its initial description in 1910. In its classic form, it usually is characterized by the mucocutaneous triad of abnormal skin pigmentation, nail dystrophy, and leukoplakia. A wide spectrum of features affecting every system (cutaneous, dental, gastrointestinal, neurological, ophthalmic, pulmonary, and skeletal) in the body, particularly the BM, have been associated with DC. Three modes of inheritance have been recognized: X-linked recessive, autosomal dominant, and autosomal recessive. The clinical phenotype associated with each of these genetic forms can vary widely. The main causes of mortality in DC are BM failure (~60%-70%), pulmonary disease (~10%-15%), and malignancy (~10%).

Clinical features of classic DC often appear in childhood. The abnormal skin pigmentation and nail changes usually appear first, often below the age of 10 years, and then BM failure develops frequently below the age of 20 years with up to 80% of patients showing signs of BM failure by the age of 30 years. But there is considerable variation between patients with respect to age of onset and disease severity even within

the same family. This causes difficulty in making a diagnosis. 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 its genetics.

The minimal clinical criteria for diagnosis of DC includes the presence of at least two out of the four major features (abnormal skin pigmentation, nail dystrophy, leukoplakia, and BM failure) and two or more of the other somatic features known to occur in DC.

Pathophysiology and link to other diseases

Since 1998, nine DC genes have been identified and these account for ~60% of DC cases. Figure 15-4 shows the different components of the telomerase and shelterin complexes with specific reference to mutations that have been associated with DC and related disorders.

X-linked DC and the Hoyeraal-Hreidarsson syndrome

The gene (*DKC1*) responsible for X-linked DC was mapped to Xq28 in 1986 and identified through positional cloning in 1998. The *DKC1* gene is highly conserved and encodes the protein dyskerin. With the identification of mutations in *DKC1*, the first diagnostic tests became available. It also provided the first firm evidence that DC was not a homogenous disorder and that other syndromes with overlapping presentation can share the same genetic mutations. The first such example was the Hoyeraal-Hreidarsson (HH) syndrome, a severe multisystem disorder, which is characterized by growth retardation of prenatal onset, microcephaly, cerebellar hypoplasia, AA, and immunodeficiency. Because of the overlap in features, it was suggested and subsequently proved that HH is a severe variant of DC because of the presence of *DKC1* mutations in males with the classical presentation of HH. *DKC1* mutations are not the only cause of HH, however; mutations in other genes also can lead to a phenotype of HH (see the following sections).

Autosomal-dominant DC and its link to telomeres and other diseases

Autosomal-dominant DC is heterogeneous; to date, heterozygous mutations in three genes (*TERC*, *TERT*, and *TINF2*) have been characterized. The identification of heterozygous mutations in *TERC* (telomerase RNA component) in 2001 was a major advance in the DC field as it provided a direct link between DC and telomerase. Telomerase is a ribonucleoprotein composed of two core components: a catalytic component, which adds the repeats; telomerase reverse transcriptase (*TERT*), and *TERC*, which acts as the template. It

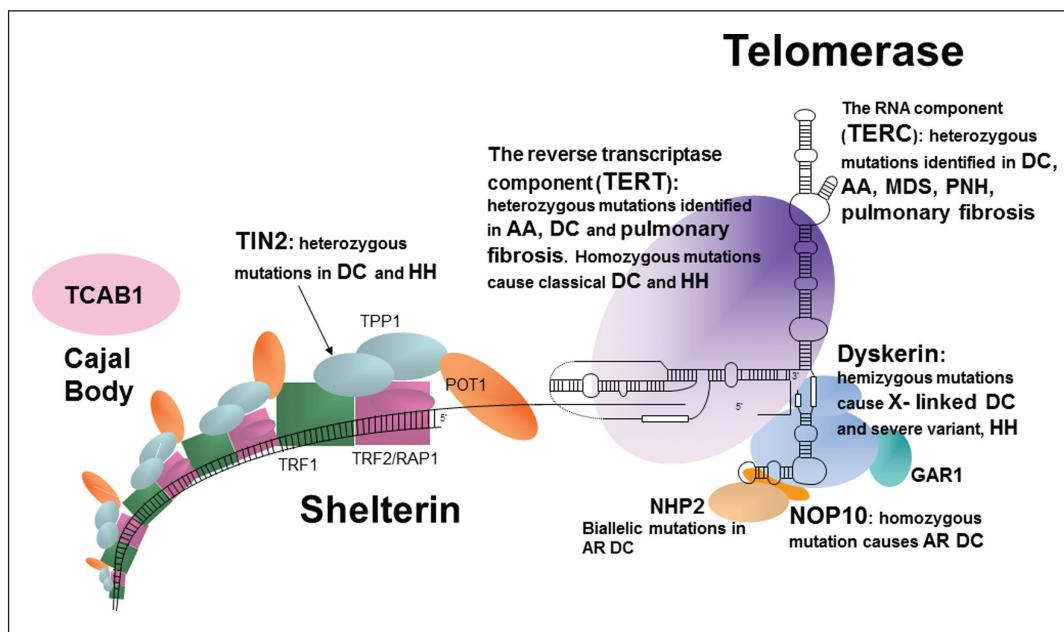


Figure 15-4 Telomeres, telomerase, and human diseases. A schematic representation of the telomerase complex (dyskerin, GAR1, NHP2, NOP10, TERC, and TERT), the shelterin complex, and their association with different categories of dyskeratosis congenita and related diseases. The telomerase complex is a RNA-protein complex because TERC is a 451b RNA molecule that is never translated. The other molecules (dyskerin, GAR1, NHP2, NOP10, and TERT) are proteins. Recent studies suggest that the minimal active telomerase enzyme is composed of two molecules each of TERT, TERC, and dyskerin. Dyskerin, GAR1, NHP2, and NOP10 are believed to be important for the stability of the telomerase complex. The shelterin complex is made up of six proteins (TIN2, POT1, TPP1, TRF1, TRF2, and RAP1) and is important in protecting the telomere. Mutations in components of the telomerase complex or the shelterin complex, as occurs in different subtypes of DC, result in telomere shortening. TCAB1 = telomere Cajal body protein 1; AA = aplastic anemia; AD-DC = autosomal dominant dyskeratosis congenita; AR-DC = autosomal-recessive dyskeratosis congenita; AR-HH = autosomal-recessive Hoyeraal-Hreidarsson syndrome; MDS = myelodysplasia; X-linked DC = X-linked dyskeratosis congenita; HH = Hoyeraal-Hreidarsson syndrome.

functions as a specialized polymerase that adds the telomeric repeat (TTAGGG) to the end of the 3' lagging strand of DNA after replication. 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. Without telomerase, the telomeres shorten with each successive round of replication, and when they reach a critical length, the cells enter senescence. Telomerase is restricted mainly to cells, such as germ cells, stem cells, and their immediate progeny, activated T-cells and monocytes; however, in cells in which telomerase is not present, telomere shortening is part of the normal process of cellular aging.

Mutations in patients with autosomal-dominant DC were identified in *TERC* initially, and it was through the identification of mutations within this molecule that led to significant expansion of the DC phenotype to include other hematological and nonhematological disorders. First, heterozygous mutations in *TERC* were identified in patients with AA and soon after in patients with MDS. This started to push the original clinical diagnosis away from the classical mucocutaneous manifestations to BM failure being the initial presenting feature.

The identification of mutations in *DKC1* and *TERC* established the pathology of defective telomere maintenance as being the principal underlying cause of DC; both dyskerin (encoded by *DKC1*) and *TERC* are now recognized to be core components of telomerase, and patients with *DKC1* and *TERC* mutations have very short telomeres compared with age-matched controls. This finding led to further study of the telomerase complex to determine the genetic basis of the remaining uncharacterized patients. The next gene to have mutations identified was *TERT*, which encodes the enzymatic component of the telomerase complex. The clinical presentation in patients with *TERT* mutations is highly variable, ranging from near DC phenotype to just AA. Heterozygous mutations in *TERT* and *TERC* have been identified in some patients with idiopathic pulmonary fibrosis, liver disease, and leukemia.

In 2008, mutations in a component (TIN2) of the shelterin complex were identified in one subtype of autosomal-dominant DC. The shelterin complex (composed of six proteins) has at least three effects on telomeres. It determines the structure of the telomeric terminus, it has been implicated in the generation of t-loops, and it controls the synthesis of telomeric DNA by telomerase. The composition and protein

interaction of the components of shelterin complex appears to be highly ordered with TIN2 playing a pivotal role. In a subset of patients with DC, HH, AA, and Revesz syndrome heterozygous mutations in the TIN2 component of shelterin have been identified. This discovery extends the range of the DC spectrum of diseases even further. Revesz syndrome is characterized by bilateral exudative retinopathy, BM hypoplasia, nail dystrophy, fine hair, cerebellar hypoplasia, and growth retardation. Patients with TIN2 mutations tend to have severe disease, and this is associated with very short telomere lengths. Interestingly, nearly all the patients have de novo TIN2 mutations, which gives rise to a different mechanism that causes the disease. In patients with heterozygous *TERC* and *TERT* mutations, studies have shown that the phenomenon of genetic anticipation frequently is involved; a parent of an affected child has the same telomerase mutation but usually no overt signs of disease. In the child with the same heterozygous telomerase mutation, however, the disease manifests itself at a much younger age and is usually more severe.

Autosomal-recessive DC

Since 2007, progress has been made in the genetic basis of autosomal-recessive (AR) DC. A large linkage study of 16 consanguineous families, including 25 affected individuals, did not identify a single common locus, suggesting there is genetic heterogeneity within this subtype of DC. Since this observation, mutations in six genes have been identified as causing AR-DC. The first AR-DC gene to be identified was *NOP10*. The homozygous *NOP10* mutation identified in a large family affected a highly conserved residue. As a result of this mutation, all the affected individuals had reduced telomere length and reduced *TERC* levels. To date, no additional *NOP10* mutations have been described. In a subset of AR-DC, biallelic mutations have been identified in *TERT*. These mutations give a different profile regarding telomerase activity and telomere length with both being greatly reduced compared with heterozygous *TERT* mutations. Biallelic mutations in *NHP2* have been identified in a third subset of AR-DC patients. Again telomere lengths and *TERC* levels are reduced in patients compared with normal controls. Both *NOP10* and *NHP2* are components of H/ACA ribonucleoprotein complex (H/ACA RNP). This complex is composed of a RNA molecule and four proteins, dyskerin, GAR1 as well as *NOP10* and *NHP2*. These four proteins are highly conserved and have been shown to be involved in ribosome biogenesis, pre-mRNA splicing, and telomere maintenance. Mutations have been identified in all components of this H/ACA RNP complex in patients with DC except for GAR1.

In 2011, biallelic mutations in the *TCAB1* gene were identified in two patients with AR-DC. *TCAB1* is a telomerase holoenzyme protein that facilitates trafficking of telomerase

to Cajal bodies, the nuclear sites of nucleoprotein complex modification and assembly. Compound heterozygous mutations in *TCAB1* disrupt telomere localization to Cajal bodies, resulting in misdirection of telomerase RNA to nucleoli. This in turn prevents telomerase from elongating telomeres, thereby resulting in short telomeres.

In another subgroup of AR-DC, biallelic mutations have been found in the *C16orf57* gene. Mutations in this gene have unified this subgroup of DC with patients classified as having poikiloderma with neutropenia and Rothmund-Thomson syndrome. This subgroup of AR-DC patients appear to have normal-length telomeres and therefore represent a biologically different subtype to the other characterized DC subgroups. The precise function of the *C16orf57* gene remains unknown. Clinically, patients with *C16orf57* mutations can be identical or similar to those with genetic defects in telomere length maintenance. Further studies are needed to establish the precise biology of this group.

In 2012, biallelic mutations in *CTC1*, encoding conserved telomere maintenance component 1, were identified in a rare subgroup of DC patients. As *CTC1* mutations initially were identified in the pleotropic syndrome Coats plus (characterized by retinopathy, intracranial calcifications and cysts, osteopenia, and gastrointestinal abnormalities), this observation expands the complexity of phenotypes associated with the “telomereopathies.” Patients with biallelic *CTC1* mutations usually tend to have nonsevere pancytopenia.

Treatment

BM failure is the main cause of premature mortality in DC. Use of the anabolic steroid oxymetholone can produce improvement in the hematopoietic function. Approximately two-thirds of patients with DC will respond to oxymetholone; 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 oxymetholone/kg/day and this can be increased, if necessary, to 2–5 mg/kg/day. It is important to monitor for side effects (eg, liver toxicity). It is possible to maintain reasonable blood counts by this approach in many patients.

The only long-term cure for the hemopoietic abnormalities is allogeneic HSC transplantation, but this is not without risk. Significant mortality is associated with BM transplants for DC patients than with other BM failure syndromes. One of the main reasons for this is the high level of pulmonary and vascular complications that present in these patients probably as a result of the underlying telomere defect. The conditioning regimen appears to have an impact on patient survival. The standard myeloablative conditioning regimes are associated with frequent and severe adverse effects, such as pulmonary complications and veno-occlusive disease.

Recently, 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 more effective treatment for DC. As in FA, DC patients need to be followed up long term for nonhematological complications.

Key points

- DC is a marrow failure syndrome classically characterized by the triad of dystrophic nails, reticular skin rash, and oral leukoplakia.
- Nonhematologic clinical features usually develop later in life and may be absent in young children.
- Nonhematologic features of DC may be mistaken for chronic GVHD in patients who received marrow transplants for AA.
- DC is associated with an increased risk for MDS, AML, and squamous cell carcinomas.
- DC is associated with genetic defects in telomere maintenance. Very short telomere lengths typically seen in these patients.
- Nine DC genes (*DKC1*, *TERC*, *TERT*, *NOP10*, *NHP2*, *TINF2*, *C16orf57*, *TCAB1*, and *CTC1*) have been identified and these account for ~60% of DC patients.
- The clinical presentation can range from AA alone to severe forms, such as the HH syndrome.

Shwachman-Diamond syndrome

Clinical features

Shwachman and Bodian and their colleagues reported this disease independently in 1964. It is now recognized as an AR disorder characterized by exocrine pancreatic insufficiency (100%), BM dysfunction (100%), and other somatic abnormalities (particularly involving the skeletal system). Signs of pancreatic insufficiency (malabsorption, failure to thrive) are apparent early in infancy (pancreatic function can improve in a subset of Shwachman-Diamond syndrome [SDS] patients by 5 years of age). Other common somatic abnormalities include short stature (~70%), protuberant abdomen, and an ichthyotic skin rash (~60%). Metaphyseal dysostosis is seen on radiographs in ~75% of patients. Other abnormalities include hepatomegaly, rib or thoracic cage abnormalities, hypertelorism, syndactyly, cleft palate, dental dysplasia, ptosis, and skin pigmentation.

The spectrum of hematological abnormalities includes neutropenia (~60%), other cytopenias (~20% have pancytopenia), myelodysplasia, and leukemic transformation (~25%). The age at which leukemia develops varies widely from 1 to 43 years. AML is the commonest category, and there is an unexplained preponderance of cases of leukemia in males (M:F ratio ~3:1).

Exocrine pancreatic insufficiency and hematological abnormalities are also seen in Pearson syndrome (PS), and this is therefore an important differential diagnosis. In PS, the anemia is usually more prominent than neutropenia and the marrow usually shows ringed sideroblasts along with vacuolation of myeloid and erythroid precursors. In addition, acidosis, abnormalities of liver function, and mitochondrial DNA rearrangements are seen in PS. PS has a worse prognosis than SDS, with many patients dying before the age of 5 years from liver or marrow failure. Other differential diagnoses to be excluded are cartilage hair syndrome and cystic fibrosis.

Pathophysiology

The SDS gene (*SBDS*) on 7q11 was identified in 2003. The majority (~ 90%) of SDS patients have been found to have biallelic mutations in this gene. Recent data from different sources provides compelling evidence that the SBDS protein has an important role in the maturation of the 60S ribosomal subunit (Figure 15-5). SDS therefore can be regarded as a disorder of ribosome biogenesis.

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. As in other cases of BM failure, supportive treatment with red cell and platelet transfusions and antibiotics is very important. The main causes of death are infection or bleeding.

Recent analysis of SDS patients has showed that the incidence of myelodysplasia and transformation to AML (~15%-25%) is higher than reported previously. The development of leukemia, often with features of myelodysplasia, usually has a poor prognosis. SDS patients with leukemia treated with conventional courses of chemotherapy usually fail to regenerate normal hematopoiesis. As this is a constitutional disorder, all somatic cells, including HSCs, are abnormal. In addition, the hemopoietic stem cells may have accumulated secondary abnormalities as suggested by complex karyotypes (especially involving chromosome 7) often observed in the BM from such patients. Therefore, for those who develop leukemia, the only approach likely to be successful is allogeneic SCT using low-intensity conditioning regimens that include fludarabine. The similarities between SDS and the other common inherited BM failure syndromes emphasize that SDS should be regarded as a disorder with high propensity to develop both AA and leukemic transformation, particularly AML with erythroid differentiation (AML-M6). As these complications may not develop until adult life, it is important to continue close hematological follow-up

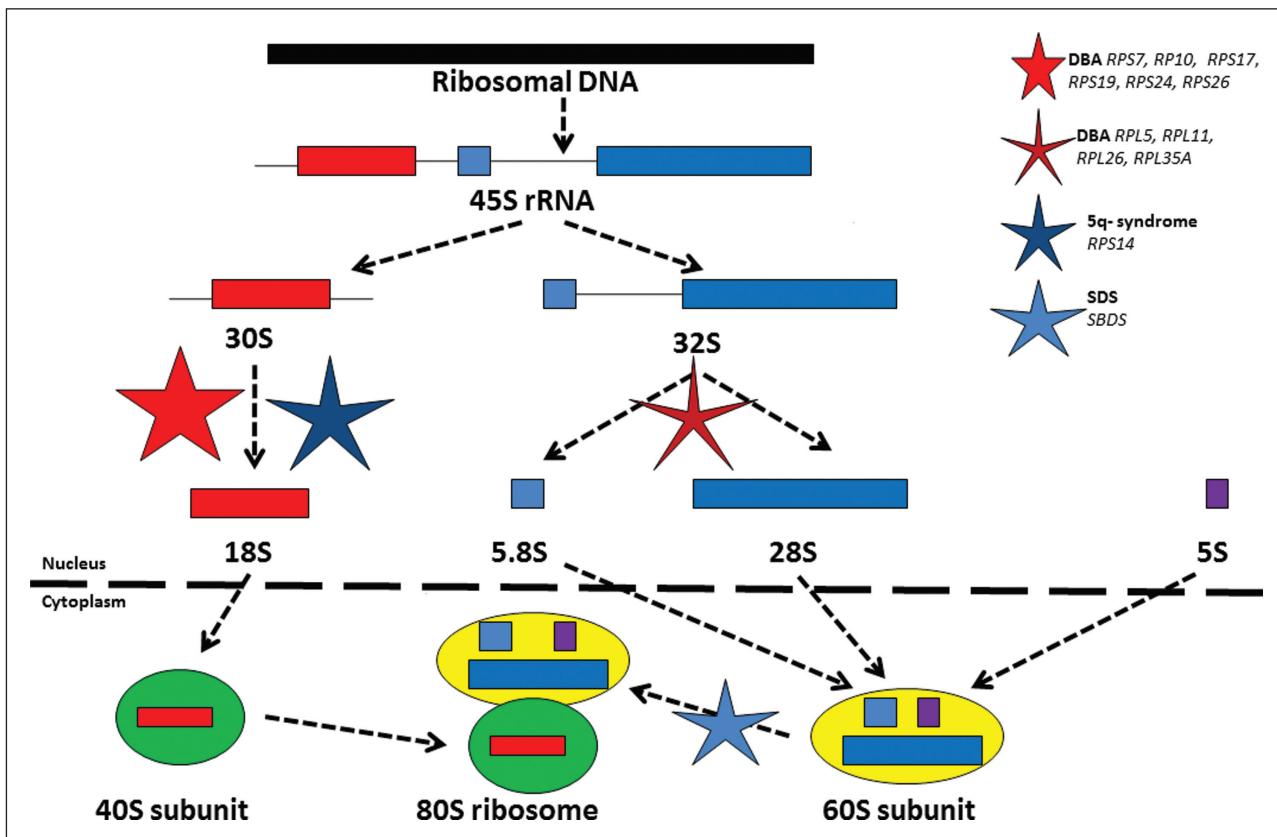


Figure 15-5 Ribosome biogenesis. Schematic showing scheme of rRNA processing in human cells and the points at which this possibly is disrupted in the different bone marrow failure syndromes. The ribosomal RNAs (rRNAs) are transcribed by RNA polymerase I as a single precursor transcript (45S rRNA). The 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 (biallelic mutations in *SBDS*), Diamond-Blackfan anemia (due to heterozygous mutations in *RPS7*, *RPS10*, *RPS17*, *RPS19*, *RPS24*, *RPS26*, *RPL5*, *RPL11*, *RPL26*, and *RPL35A*) and 5q- syndrome (haploinsufficiency of *RPS14*) are indicated by the different colored stars. DBA = Diamond-Blackfan anemia; SDS = Shwachman-Diamond syndrome.

throughout life. Nonhematological malignancies have not been observed in SDS patients.

Key points

- SDS is a rare autosomal-recessive disorder characterized by BM failure and exocrine pancreatic insufficiency.
- The majority of SDS patients have biallelic mutations in the *SBDS* gene, which has an important role in ribosome biogenesis.
- Like other BM failure syndromes, SDS patients 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.

Pearson syndrome

PS is a multisystem mitochondrial disease originally described as a disorder with sideroblastic anemia, vacuolization of hematopoietic precursors, and exocrine pancreas

dysfunction. Other features include hypoplastic macrocytic anemia alone or associated with thrombocytopenia or granulocytopenia, proximal tubular insufficiency, failure to thrive, endocrine pancreatic insufficiency with insulin-dependent diabetes, lactic acidosis, hyperlipidemia with liver steatosis, muscle and neurologic impairment, and, frequently, early death (usually in infancy). Treatment is supportive. The few patients who survive into adulthood often develop neurological symptoms of Kearns-Sayre syndrome.

The BM cellularity can be variable and all cell lineages may be affected; the anemia is usually macrocytic with prominent vacuoles in cells of both erythroid and myeloid lineages. Ringed sideroblasts can be identified in a subset of PS patients. Hematopoietic dysfunction associated with peripheral cytopenias is a major cause of morbidity and mortality.

Although the syndrome was first described by Pearson et al. in 1979, the mitochondrial DNA deletions causing it were discovered a decade later in 1989. The mitochondrial DNA deletions can vary in size from patient to patient. These lead

to loss of mitochondrial function (defective oxidative phosphorylation). As cells contain many mitochondria, normal and mutant mitochondria can coexist in the same cell; the proportion of which can vary from tissue to tissue (heteroplasmy). The extent to which different tissues are affected depends in part on this proportion. This explains why different tissues may be affected to varying degrees in the same patient.

Key point

- PS is a multiorgan mitochondrial DNA disorder characteristically associated with marrow and pancreas dysfunction.
Treatment is largely supportive and many patients die in infancy.

Diamond-Blackfan anemia

Clinical features

DBA classically presents within the first year of life with a hypoproliferative, macrocytic anemia. BM examination typically reveals a paucity of erythroid precursors. Approximately 50% of patients have associated nonhematologic physical findings, which may include radial ray anomalies, midline craniofacial defects or cleft palate, urogenital abnormalities, or cardiac defects. Red blood cell adenosine deaminase levels and fetal hemoglobin levels usually are elevated, and this can help in diagnosis. An increased risk of AML has been reported in DBA patients, but the risk is less than in patients with FA.

Pathophysiology

Heterozygous germline mutations in the *RPS19* gene, which encodes a ribosomal protein, have been reported in 25% of patients with DBA. The clinical manifestations among family members with identical *RPS19* gene mutations are highly variable, and anemia may be absent. It is not clear how a defect in one 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 cellular protein synthesis broadly. Intact ribosomes appear to be particularly important for normal erythropoiesis; in patients with 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.

Germline mutations in nine other genes encoding ribosome-associated proteins (*RPS7*, *RPS10*, *RPS17*, *RPS24*, *RPS26*, *RPL5*, *RPL11*, *RPL26*, and *RPL35A*) have been described in families with DBA who have wild-type *RPS19*. Although the overall inheritance pattern is autosomal

dominant, many cases of DBA appear to be sporadic, presumably resulting from new germline mutations. Recently, constitutional heterozygous *GATA1* mutations have been identified in some patients with “DBA-like” disease.

Treatment

In the majority of patients with DBA (~80%), the hemoglobin level improves with corticosteroid treatment. It is vital to use the minimal dose of steroids required to support erythropoiesis to minimize adverse effects of chronic steroid use. Patients who fail to respond to steroids, or who require high steroid doses, may be supported with red blood cell transfusions instead. Careful attention to iron overload and timely initiation of iron chelation therapy are important for patients undergoing chronic transfusions.

Currently, the only curative treatment of marrow failure is stem cell transplantation, but the risks of transplantation must be weighed against the benefits for each patient. Some patients with DBA will undergo spontaneous remission and maintain adequate hemoglobin levels independent of steroids. In a few patients, a diet supplemented with leucine and isoleucine has resulted in improved erythropoiesis and growth; this approach needs to be investigated further.

Key points

- DBA typically presents with macrocytic anemia and red blood cell hypoplasia in infancy.
- Approximately 50% of patients with DBA have physical signs, including radial ray and craniofacial abnormalities.
- Autosomal-dominant mutations in 10 different ribosomal genes account for ~60% of DBA patients.
- Treatment options for DBA include corticosteroids, red blood cell transfusion support, and stem cell transplantation.
- Spontaneous remissions may occur in a subset of patients.

Congenital dyserythropoietic anemia

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, but CDA III is poorly understood, 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, and these should be ruled out.

CDA type I

CDA I is a rare autosomal-recessive disorder (~80 pedigrees are listed in the pan-European registry) that usually presents in childhood or adolescence. CDA is characterized by hemolytic anemia (usually moderate, with hemoglobin in the range of 9–10 g/dL), anisopoikilocytosis with reduced levels of erythrocyte membrane protein 4.1R, normal or elevated reticulocyte count, macrocytosis, and high serum iron levels. Serum bilirubin levels often are elevated, and some patients develop pigment gallstones or jaundice. Splenomegaly is a frequent feature. Some patients have dysmorphologic features, including syndactyly, absent or hypoplastic distal phalanges or nails, skin pigmentation abnormalities, hypoplastic right third rib, and sensorineural deafness. BM examination shows erythroid hyperplasia, binucleated erythroblasts, and a distinctive pattern of internuclear chromatin bridging.

CDA I has been linked to defects in the gene codanin 1 (*CDAN1*) at chromosome 15q15, a gene encoding a protein of unknown function and without well-defined domains. At least one kindred with CDA I lacked *CDAN1* mutations, and linkage analysis failed to connect the condition in this pedigree to chromosome 15q, so another gene also may be responsible in some cases.

For unclear reasons, the anemia in type I CDA typically responds to recombinant interferon- α . Folate supplementation is helpful, given the chronic hemolysis. Most patients with type I CDA typically do not require transfusions, and transfusions can exacerbate the tendency to iron overload.

CDA type II

CDA II is more common than CDA I (~340 patients have been collected in European registries) and usually presents during childhood with anemia of variable severity. 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. Cases of CDA II have been linked to chromosome 20q11. In 2009, the gene encoding the secretory COPII component SEC23B was shown to be mutated in CDAII.

CDA II formerly was known as HEMPAS (hereditary erythroblastic multinuclearity with a positive acidified serum test). A characteristic feature of HEMPAS is the ability of

some group-compatible sera to lyse the patient's erythrocytes, resulting in a positive acid hemolysis test (Ham test). The sucrose hemolysis test is negative, however, and the cells are not lysed by autologous serum, unlike the situation in paroxysmal nocturnal hemoglobinuria (PNH). The cell lysis is secondary to increased immunoglobulin M (IgM) binding to erythrocytes and not to increased sensitivity to complement.

Like other patients with congenital dyserythropoiesis, patients with CDA II can have problems with iron overload. Phlebotomy and iron chelation have been used to treat iron overload in these patients. 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. The causative gene is unknown, but in one kindred, the disorder was linked to chromosome 15q21. The peripheral smear shows marked anisopoikilocytosis and basophilic stippling of the red blood cells, a picture similar to β -thalassemia major. Additionally exceptionally rare types of CDA (eg, CDA IV, V, VI, and VII) also have been proposed. Recently a unique mutation in *KLF1*, which encodes the erythroid transcription factor KLF1, was identified; this causes major ultrastructural abnormalities (a hitherto unnamed CDA), the persistence of embryonic and fetal hemoglobins, and the absence of some red cell membrane proteins.

Key points

- CDA I is characterized by moderate hemolytic anemia, internuclear chromatin bridging, iron overload, germline mutations in *CDAN1*, 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 a positive acid hemolysis (Ham) test, multinucleated giant cells, and a low reticulocyte count. The genetic defect in this subtype is in the *SEC23B* gene.
- Several rare forms of CDA have not yet been genetically characterized.

Congenital and cyclical neutropenia

Congenital neutropenia is a heterogeneous disorder. It includes Kostmann syndrome, which first was described in

1954. Although the original description by Kostmann was of an autosomal-recessive disorder, other congenital neutropenia subtypes (both sporadic and autosomal dominant) have been included subsequently in this category. The neutropenia usually is recognized at birth and the neutrophil count is often $<0.2 \times 10^9/L$. The Hb and platelet count are usually normal and the BM shows maturation arrest of myelopoiesis at the level of the promyelocyte/myelocyte state (with abundant promyelocytes but with a selective reduction in myelocytes, metamyelocytes and neutrophils).

The neutropenias are associated with severe infections and early death. No patient has developed AA, but myeloid leukemias (~25% by 25 years) can occur. The availability of G-CSF has revolutionized the outcome of these children. Somatic mutations in the gene that encodes the G-CSF receptor have been documented during the evolution to leukemia in patients receiving G-CSF. The precise contribution of G-CSF therapy to the development of G-CSF receptor mutations remains unclear. For patients who become refractory to G-CSF or who develop leukemia, SCT may be appropriate and curative.

Cyclical neutropenia is characterized by a neutrophil count that usually reaches a nadir with a 21-day periodicity. Around the nadir, patients may develop fever and mouth ulcers. In cyclical neutropenia, the pattern of inheritance is usually autosomal dominant. Linkage analysis in affected families resulted in the localization of the disease gene to 19p13.3. Subsequent studies identified mutations in the gene (*ELA2*) encoding neutrophil elastase (NE). An extraordinary twist was the identification of *ELA2* mutations in many patients who also had congenital neutropenia. In cyclical neutropenia, the mutations usually are clustered around the active site of the molecule, whereas the opposite face of the molecule tends to be mutated in congenital neutropenia patients. NE is a serine protease that is synthesized predominantly at the promyelocytic stage and can be expected to be important in neutrophil development. Recent studies suggest that *ELA2* mutations lead to accumulation of the non-functional protein, which in turn triggers an unfolded protein response (UPR) leading to maturational arrest. The precise mechanism leading to maturation arrest of promyelocytes remains unclear. The original family described by Kostmann, had autosomal-recessive severe congenital neutropenia (SCN), and recently has been shown to be associated with biallelic mutations in the *HAX1* gene predicted to lead to defects in cell death. Biallelic mutations in *HAX1* account for ~10% of congenital neutropenia. The *HAX1* protein is a critical regulator of the mitochondrial membrane potential and cellular viability. Although data suggest *HAX1* is important in controlling apoptosis it is unclear why premature death of neutrophils consistently is associated with *HAX1* deficiency. Mutations in other genes (*GFI1*,

G6PC3, *WASP*) are known to be rarely associated with SCN, demonstrating genetic heterogeneity.

Key points

- SCN is a heterogeneous disorder, and several disease genes (*ELA2*, *HAX1*, *GFI1*, *G6PC3*) have been identified to date.
- G-CSF has become an important therapeutic agent for these patients. Patients who become refractory to G-CSF or progress to leukemia can be treated by HSCT.

WHIM syndrome

WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome is a rare autosomal-dominant disorder characterized by neutropenia, B-cell lymphopenia, hypogammaglobulinemia, and extensive human papillomavirus (HPV) infection. Affected individuals typically present with recurrent bacterial (eg, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Klebsiealla pneumoniae*, *Staphylococcus aureus*) infections from birth with ANCs of 1,000/ μ L. Recurrent pneumonias may in some cases lead to severe bronchiectasis. Despite the peripheral neutropenia, the BM of affected patients is generally hypercellular with increased numbers of mature neutrophils (a condition termed *myelokathexis*). Patients commonly have B-cell lymphopenia, yet immunity to most viral pathogens is normal. The major exception is HPV, which is the cause of warts in patients with WHIM syndrome. Although some patients have few, if any, warts, the majority of patients suffer from extensive verrucosis. They typically appear in the first or second decades of life and can involve any mucocutaneous surface. Hypogammaglobulinemia is variable, ranging from normal to modestly decreased serum immunoglobulin (Ig) G, IgM, and IgA. Treatment with G-CSF or GM-CSF is effective in correcting the neutropenia; those with significant hypogammaglobulinemia benefit from intravenous immunoglobulin (IVIg) therapy every 6 weeks.

The majority of patients with WHIM syndrome have heterozygous mutations of the *CXCR4* gene. *CXCR4* is a G protein-coupled heptahelical receptor that is the major receptor for SDF1 (*CXCL12*). Evidence is convincing that SDF1/CXCR4 signaling is a key regulator of neutrophil release from the BM. Specifically, SDF1/CXCR4 provides a signal to retain neutrophils in the BM. The mutations of *CXCR4* in WHIM syndrome truncate the carboxyl-terminal (cytoplasmic) tail, resulting in enhanced CXCR4 signaling. These observations support the current model in which enhanced signaling by the *CXCR4* mutants in WHIM syndrome leads to abnormal neutrophil retention in the BM. Given that many of the clinical features affecting WHIM

patients are a consequence of hyperfunction of CXCR4, inhibitors of CXCR4 function, such as plerixafor, are being investigated in clinical trials with some therapeutic promise.

Key points

- WHIM syndrome is a congenital immune deficiency characterized by susceptibility to HPV infection-induced warts, neutropenia (associated with BM myelokathexis), B-cell lymphopenia and hypogammaglobulinemia-related recurrent infections.
- The syndrome is due to heterozygous mutations in the CXCR4 gene, resulting in functional overactivity of CXCR4.
- Clinical management includes therapy with G-CSF, IVIg, prophylactic antibiotics, and surveillance or surgical removal of dysplastic skin or mucosal HPV-related lesions.

Thrombocytopenia with absent radii

Thrombocytopenia with absent radii (TAR) is an autosomal-recessive disorder characterized by hypomegakaryocytic thrombocytopenia and bilateral radial aplasia. 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 $<50 \times 10^9/\text{L}$. The leucocyte count can be normal or raised sometimes up to $100 \times 10^9/\text{L}$ (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. The mainstay of management is prophylactic and therapeutic use of platelet transfusions. TAR patients have a good prognosis after infancy. There have been no reports of AA.

In TAR patients, thrombopoietin levels usually are 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 (MPL). Recently, it has been determined that compound inheritance of low-frequency regulatory SNP and a rare null mutation in the *RBM8A* gene (which encodes a subunit of the exon-junction complex) causes TAR.

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.
- It recently was established that TAR is due to biallelic mutations in the *RBM8A* gene, which encodes a subunit of the exon-junction complex.

Congenital amegakaryocytic thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare autosomal-recessive disorder characterized by amegakaryocytic thrombocytopenia. Megakaryocytes are absent or greatly diminished in the BM. Patients typically present shortly after birth with petechiae, bruising or bleeding, and a very low platelet count. Patients with CAMT frequently progress to pancytopenia associated with BM aplasia (AA). There are also case reports of patients with CAMT developing MDS and leukemia.

CAMT is caused by biallelic mutations in the *MPL* gene, which encodes the thrombopoietin (TPO) receptor. Development of AA in CAMT patients 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. Many patients progress to aplasia, and this can be cured with HSCT. The platelet count is not responsive to TPO.

Key points

- CAMT is caused by autosomal-recessively inherited defects 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.

Chédiak-Higashi syndrome

Chédiak-Higashi syndrome (CHS) is a rare inherited syndrome characterized by severe immunodeficiency, partial albinism, a mild bleeding diathesis, and progressive neurologic defects. The pathognomonic feature of CHS is the presence of giant inclusion bodies in virtually all granulated cells, particularly neutrophils (Figure 15-2). Neutropenia is common,

and the residual neutrophils display functional defects. Approximately 85% of patients will progress to an accelerated phase characterized by a nonclonal lymphohistiocytic infiltration of multiple organs, leading to multiorgan system failure. CHS is inherited in an autosomal-recessive fashion and is due to mutations of the *LYST* gene. The loss of LYST protein disrupts vesicular trafficking, leading to impaired formation of secretory lysozymes and resulting in hypopigmentation and dysregulated immune cell function. Of note, there are several other rare neutropenic disorders in which impaired vesicular trafficking appears to be the primary mechanisms of disease pathogenesis (Tables 15-3 and 15-4). Other than allogeneic BM transplantation, treatment of CHS and related syndromes is largely supportive.

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 3–28 weeks (average of 11 weeks). Overall, the prognosis for infants with alloimmune neutropenia syndrome generally is favorable.

Primary autoimmune neutropenia

Primary autoimmune neutropenia (also termed chronic benign neutropenia of infancy and childhood) 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/ μ L. 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. In the largest series, 95% of 240 patients with autoimmune neutropenia had a spontaneous remission, 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. 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 have 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 (LGL) 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 but are present in some SLE patients without neutropenia, 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, long-standing 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, monocytopenia, 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. 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 for 1 year 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. Within a few minutes, a complete blood count (CBC) reveals a white blood cell (WBC) count of 1.5'109/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.

Drug-induced neutropenia and agranulocytosis are serious medical problems, with an estimated frequency of 1 per 1.6-7 million and a case fatality rate of approximately 5%. Agranulocytosis refers to the complete absence of neutrophils in the blood. Although certain medications carry a higher risk of neutropenia (Table 15-5), it is probably 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 recent systematic review of the literature identified 10 drugs that accounted for ≈50% of cases of definite or probable reports of drug-induced neutropenia: carbimazole, clozapine, dapsone, dipyrone, methimazole, penicillin G, procainamide, propylthiouracil, rituximab, sulfasalazine, and ticlopidine. In most cases, agranulocytosis presents within 6 months, and usually within 3 months, after starting the offending drug. The clinical presentation of drug-induced agranulocytosis is often less dramatic than in this case, but patients often have fever and pharyngitis as their first symptoms. Sepsis or pneumonia may occur in 10%-30% of patients. Usually the prognosis is good because neutrophil counts recover within approximately 1 week if the offending medication is withdrawn. The disease mechanism often is unclear. 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. Serial blood counts are now recommended for patients on some drugs (ie, sulfasalazine, clozapine, phenothiazines, and antithyroid drugs) because of the relatively high frequency of drug-induced neutropenia associated with these agents.

Table 15-5 Selected drugs associated with neutropenia.

Anti-inflammatory agents	Antimicrobial agents
Aminopyrine*	Ampicillin*
Diclofenac*	Cefotazime*
Diflunisal*	Cefuroxime*
Dipyrone*	Flucytosine*
Ibuprofen*	
Gold salts	Fusidic acid*
Penicillamine	Imipenem-cilastatin*
Phenylbutazone	Nafcillin*
Sulfasalazine	Oxacillin*
	Quinine*
Cardiovascular agents	Ticarcillin*
Clopidogrel*	Chloramphenicol
Disopyramide*	Sulfomamides
Methyldopa*	Amodiaquine
Procainamide*	Dapsone
Quinidine*	Terbinafine
Ramipril*	Vancomycin
Spironolactone*	
Dipyridamole	Antithyroid agents
Captopril	Propylthiouracil*
Ticlopidine	Carbimazole
	Methimazole
Anticonvulsants	
Phenytoin*	Other agents
Carbamazepine	Amygdalin*
	Calcium dobesilate*
Psychotropic agents	Cimetidine*
Chlorpromazine*	Infliximab*
Clozapine*	Levamisole*
Fluoxetine*	Metoclopramide*
Mianserin	Mebhydrolin*
	Rituximab
Hypoglycemic agents	Ranitidine
Chlorpropamide	Famotidine
Tolbutamide	Metiamide
Glyburide*	

*Level I evidence based on Andersohn et al. *Annals of Internal Medicine*. 2007; 146:657.

Documentation of the role of specific drugs in the causation of neutropenia depends on (i) the frequency of the occurrence among patients; (ii) the timing of the event in relationship to drug use; (iii) the absence of alternative explanations; and (iv) the inadvertent or intentional reuse of the drug (rechallenges) with a similar response.

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. 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. Overall survival is ~95%. A neutrophil count $\leq 0.1 \times 10^9/L$ and the presence of sepsis or severe infection are associated with delayed neutrophil recovery and increased mortality.

Recently agranulocytosis has been associated with both cocaine and heroin use. In reported cases, it was caused by the adulteration of the drug with levamisole, an antihelminthic drug used in veterinary medicine that is known to be associated with agranulocytosis.

Key points

- Transient neutropenia is not uncommon 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 is due to infection, acquired hematopoietic disease, autoimmune disorder, or a clonal proliferation of large granular lymphocytes.

Congenital disorders of neutrophil function

Clinical case

A 2-year-old boy 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.

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 and requires a careful workup. Certain conditions, however, should raise concern for an underlying immunodeficiency syndrome, including neutrophil function disorders, and may merit further evaluation. These include the following: (i) recurrent systemic bacterial infections (eg, sepsis, osteomyelitis, meningitis); (ii) infections at unusual sites (eg, hepatic or brain abscess); (iii) recurrent bacterial infections (eg, pneumonia, sinusitis, cellulitis, lymphadenitis, draining otitis media); (iv) infections caused by unusual pathogens (eg, *Aspergillus* pneumonia, disseminated candidiasis, *Serratia marcescens*, *Nocardia* species, *Burkholderia cepacia*); and (v) 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. The most widely known disorders with abnormalities of neutrophil function are described in the following sections.

Myeloperoxidase 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 for MPO on 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.

Leukocyte adhesion deficiency

Leukocyte adhesion deficiency (LAD) is a rare disorder manifested by delayed wound healing, recurrent bacterial infections, and neutrophilia. There are three distinct forms of LAD. In LAD-I, mutations of *ITGB2*, encoding the β_2 -integrin (CD18) chain, disrupt β_2 -integrin function. In LAD-II, mutations of *FUCT2*, encoding GDP-fucose transporter-1, disrupt the generation of ligands on neutrophils required for selectin binding. Patients with LAD-II also display short stature, abnormal facies, and severe cognitive impairment. Finally, in LAD-III, mutations of *FERMT3* (*KINSLIN3*) or *RASGRP2* lead to impaired β -integrin function. In addition to immunodeficiency, patients with LAD-III also have a bleeding diathesis due to a defect in β_3 -integrin function on

platelets. All mutations result in severely impaired neutrophil chemotaxis and emigration from the blood to sites of infection. A history of consanguinity may be an important clue in evaluating children for LAD. Definitive treatment of LAD requires allogeneic HSCT, with a recent study reporting a 5-year survival of 75%.

Hyperimmunoglobulin E syndrome

Hyperimmunoglobulin E syndrome (previously known as Job syndrome) is manifested by defective neutrophil chemotaxis, recurrent bacterial infections (typically involving the skin, sinuses, or lung), mucocutaneous infections with *Candida albicans*, and elevated serum IgE levels. Patients may present with pruritic dermatitis in the first few weeks of life. Associated features that might aid in the diagnosis include coarse facial features, recurrent fractures, and short stature. Recent studies have identified mutations of *STAT3* in the majority (60%-70%) of cases of hyperimmunoglobulin E syndrome. In addition, mutations of *DOCK8* (encoding dedicator of cytokinesis 8) are present in many cases of the autosomal-recessive form of hyperimmunoglobulin E syndrome.

Chronic granulomatous disease

Chronic granulomatous disease (CGD) is a primary immunodeficiency syndrome caused by a defect in 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 and other tissue. The majority of cases are diagnosed in early childhood. Infections are caused by catalase-positive organisms, with the majority of infections being caused by *Aspergillus* species, *Burkholderia* species, *S. aureus*, *Nocardia* species, and *Mycobacteria* species. The mutations responsible for CGD occur in one of the components of the NADPH oxidase system and 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 gp91^{phox} component of the membrane cytochrome b₅₅₈ protein complex. The other cases involve mutations of autosomal genes, including *CYBA*, which encodes p22phox (the second membrane component of cytochrome b558) (5% of CGD cases); NCF-1, which encodes p47phox (20% of cases); and NCF-2, which encodes p67phox (6% of cases). The incidence is approximately 1 in 200,000 live births.

The diagnosis of CGD is established by a typical clinical history and a laboratory test demonstrating an abnormal

neutrophil oxidative burst. In the nitroblue tetrazolium (NBT) test, neutrophils are incubated with NBT. Patients with CGD are unable to generate superoxide and fail to reduce NBT to a blue formazan precipitate. In the dihydrorhodamine assay, oxidation of dihydrorhodamine to rhodamine by hydrogen peroxide produced by activated neutrophils is measured by flow cytometry. Genetic testing for both X-linked and autosomal-recessive variations is also available.

Treatment of CGD consists of prophylactic antibiotics, antifungal agents, and the prompt administration of antibiotics 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.

Autoinflammatory diseases

Autoinflammatory diseases, also called periodic fever syndromes, are a group of rare hereditary 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 is characterized by sporadic paroxysmal attacks of fever, serosal inflammation, and neutrophilia. The condition usually presents in early childhood with recurrent attacks of acute peritonitis, although pleuritis and synovitis also are frequent. These attacks generally last 1-3 days and then resolve spontaneously. FMF is inherited as an autosomal-recessive disorder and mainly occurs in populations from the Mediterranean basin, such as Sephardic Jews, Arabs, Turks, and Armenians. Sporadic cases, however, also have been reported in individuals of French, Italian, Greek, Belgian, and Northern European heritage. 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 by activated neutrophils. 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 AA amyloidosis, especially in the kidneys. In this regard, FMF is the leading cause of secondary amyloidosis in Turkey. 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 now can be confirmed and family studies can be carried out using molecular studies done by a reference research laboratory. Colchicine prevents clinical attacks and tissue amyloid deposition in most patients with FMF. Rare patients with exceptional, refractory disease have undergone successful HSCT.

Hyper-IgD syndrome is another rare autosomal-recessive autoinflammatory disease found in Dutch and French families that is associated with mutations in the mevalonate kinase gene *MVK*. The tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS; previously known as familial Hibernian fever) is an autosomal-dominant disorder affecting Scottish and Irish individuals and associated with mutations in the gene-encoding TNF receptor 1, *TNFRSF1A*. Cryopyrin-associated periodic syndromes (CAPS) 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 CAPS, followed by Muckle-Wells syndrome and familial cold autoinflammatory 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.

Key points

- Genetic disorders affecting neutrophil function are rare causes of recurrent infections, unexplained fever, and inflammation.
- Disorders affecting neutrophil adhesion, chemotaxis, and killing usually are diagnosed in young children with recurrent infections.
- Chronic granulomatous disease is characterized by recurrent bacterial and fungal infections and is due to mutations that impair the ability of phagocytes to generate reactive oxygen intermediates.
- FMF should be considered in children or adults with unexplained recurrent fever and inflammation and the appropriate ethnic background.

Disorders of histiocytes and DCs

Hemophagocytic lymphohistiocytosis

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 is also notable for a markedly elevated ferritin 24,000 ng/mL (normal 4-76 ng/mL) and hypofibrinogenemia of 68 mg/dL (normal 150-450 mg/dL). A BM biopsy reveals marked histiocyte hyperplasia with hemophagocytosis. She begins treatment with dexamethasone, cyclosporine, 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 15-6). The major pathophysiologic abnormality in HLH is the high production of inflammatory cytokines with abnormal T-cell activation. Severe impairment in natural killer (NK) cell activity and cytotoxic T-cell function are also characteristic of the disease.

HLH may occur either as an inherited or acquired disorder (Table 15-6). Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal-recessive disease that classically presents in infancy and early childhood with an estimated incidence of approximately 1 in 50,000. Mutations involving the *PRF1* gene, encoding perforin, have been found in up to 50% of patients with FHL. Perforin is a critical component of the granule exocytosis pathway, which enables NK cells and cytotoxic T-lymphocytes to induce apoptosis in target cells. Additional mutations in the *Munc13-4* gene (*UNC13D*), resulting in defective granule exocytosis, and in *STX11*, a t-SNARE involved in intracellular trafficking, also have been described in FHL. In addition to FHL, HLH also occurs in the context of inherited immune deficiency syndromes, including CHS, Griscelli syndrome, and X-linked proliferative syndromes.

Acquired HLH syndrome, also known as reactive hemophagocytic syndrome or secondary HLH (sHLH), can affect both adults and children and usually is associated with an

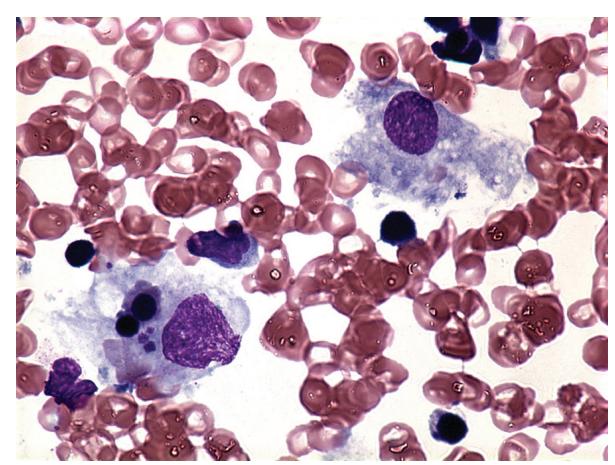


Figure 15-6 Hemophagocytic lymphohistiocytosis. Bone marrow aspirate demonstrating phagocytic histiocytes with ingested platelets and RBC precursors. From ASH Image Bank, #3502.

Table 15-6 Hereditary and acquired causes of HLH.

Primary HLH	
Familial HLH	
Chédiak-Higashi syndrome	
Griscelli's syndrome	
X-linked lymphoproliferative (XLP) disease	
Wiskott-Aldrich syndrome (WAS)	
Secondary HLH	
Infections	
Herpes virus infection	
HIV	
Parvovirus, adenovirus, hepatitis virus	
Bacteria, rickettsia, fungal, spirochete associated infections	
Malignancy	
AML, MDS, lymphomas, multiple myeloma	
Metastatic carcinoma	
Autoimmune diseases (Macrophage activation syndrome)	
Other immunodeficiency states	
Posttransplant	
Cytotoxic or immunosuppressive therapy	
Postsplenectomy	

underlying infection or other immunocompromised state. Predisposing conditions associated with sHLH include infections, autoimmune or rheumatologic disorders, hematologic and (less commonly) nonhematologic malignancies, AIDS (with or without opportunistic infections), and posttransplantation immunosuppression. The pathophysiology of sHLH appears to be similar to FLH, except that in these patients, the underlying predisposing disorder, and not a congenital defect, is 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. Laboratory findings include elevated ferritin and triglycerides with low fibrinogen. Central nervous system (CNS) involvement may range from irritability, bulging fontanel, and neck stiffness to seizures, cranial nerve palsies, ataxia, or coma. Lymphadenopathy, rash, and liver disease also may be present. A BM biopsy is crucial to identify histiocytic hyperplasia and hemophagocytosis, although hemophagocytosis may not be observed early in the clinical course. Lumbar puncture and magnetic resonance imaging (MRI) of the brain should be performed in those with suspected CNS involvement.

Although evaluation for an underlying infection should be performed, both familial and secondary forms of HLH are frequently triggered by an infection. Diagnostic criteria for HLH have been established by the Histiocyte Society

(Table 15-7). In addition to the original criteria proposed in 1991 of fever, splenomegaly, cytopenias, hypertriglyceridemia or hypofibrinogenemia, and hemophagocytosis, three additional criteria were introduced in 2004; these are low or absent NK-cell activity, hyperferritinemia, and high levels of sIL-2R. At least five of eight clinical criteria or the presence of either familial disease or one of the known genetic abnormalities is required for diagnosis of HLH.

Although sHLH may resolve after treatment of the underlying condition or with a short course of immunosuppression, untreated FHL is uniformly fatal within 1-2 months. Treatment of FHL consists of chemoimmunotherapy followed by allogeneic stem cell transplantation. The HLH-94 protocol of the Histiocyte Society for FHL consists of an initial 8 weeks of dexamethasone and etoposide followed by maintenance cyclosporine with pulses of etoposide and dexamethasone. Intrathecal therapy with methotrexate and corticosteroids is administered in individuals with evidence of CNS involvement. Results of HLH-94 demonstrate a 3-year survival rate of 51%. The subsequent protocol, HLH-2004, is similar to the HLH-94 protocol and includes etoposide, dexamethasone, and cyclosporine with an earlier introduction of cyclosporine to reduce the risk of relapse while corticosteroids are being tapered. Allogeneic HSCT is recommended in patients with FHL and in patients with relapsed or refractory sHLH.

Macrophage activation syndrome (MAS) is considered to be a variation of sHLH, which occurs in individuals with autoimmune disorders. The disorder most frequently seen is systemic juvenile idiopathic arthritis (SJIA) but also can 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 as some features, such as hyperferritinemia, lymphadenopathy, and splenomegaly, often are present during a flare of the underlying disease.

Langerhans cell histiocytosis

Langerhans cells are specialized DCs that are found in the skin and mucosa. Langerhans cell histiocytosis (LCH) is a clonal disorder of DCs associated with polymorphic cellular infiltration and damage at either unifocal tissue sites or in multiple organs and tissues. Although the DCs in LCH express similar antigens, including CD1a and CD207 as skin Langerhans cells, they are believed to originate from a

Table 15-7 2004 Revised diagnostic criteria for hemophagocytic lymphohistiocytosis.

The diagnosis HLH can be established if one of either 1 or 2 is fulfilled:

1. A molecular diagnosis consistent with HLH
2. Diagnostic criteria for HLH fulfilled (five out of the following eight criteria)
 - A. Initial diagnostic criteria (to be evaluated in all patients with HLH)
 - 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 × 10⁹/L
 - Neutrophils <1.0 × 10⁹/L
 - Hypertriglyceridemia and/or hypofibrinogenemia:
 - Fasting triglycerides ≥3.0 mmol/L (ie, ≥265 mg/dL)
 - Fibrinogen ≤1.5 g/L
 - Hemophagocytosis in bone marrow or spleen or lymph nodes
 - No evidence of malignancy
- B. New diagnostic criteria
 - 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

If hemophagocytic activity is not proven at the time of presentation, further search for hemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material may be obtained from other organs. Serial marrow aspirates over time may also be helpful.

The following findings may provide strong supportive evidence for the diagnosis: (i) spinal fluid pleocytosis (mononuclear cells) or elevated spinal fluid protein, (ii) histological picture in the liver resembling chronic persistent hepatitis (biopsy).

Other abnormal clinical and laboratory findings consistent with the diagnosis are cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, skin rash. Hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, increased very-low density lipoprotein (VLDL), decreased high density lipoprotein (HDL).

distinct myeloid Dc precursor. Historically, localized LCH was referred to as eosinophilic granuloma, whereas clinical variants of multisystem disease were referred to as histiocytosis X, Letterer-Siwe disease, and Hand-Schüller-Christian syndrome. Recently, mutations in BRAF (V600E) have been identified in >50% of patients LCH.

Patients with LCH are categorized as having either uni- or multifocal involvement of a single organ system (SS-LCH) or multisystem LCH (MS-LCH). SS-LCH most commonly involves the bone (particularly the skull, femur, pelvis, and ribs) and less commonly involves the skin, lymph nodes, and lung. Usual presentations of limited disease include persistent or recurrent and progressive bony pain or swelling, chronic skin rash, chronic ear drainage, dyspnea, cough, and pneumothorax (isolated bony involvement resulting in ear drainage is more common in children, and pulmonary disease occurs predominantly in adults). 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. 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. LCH is rare, with an annual incidence of approximately 5 per million. The etiology of LCH is unknown, although LCH of the lungs in adults frequently is associated with smoking.

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 15-7). Langerhans cells are positive for CD1a and S-100, and by ultrastructural examination, contain the hallmark Birbeck granules. Birbeck granules are tennis racket-shaped cytoplasmic granules ~200-400 nm in length and 33 nm in width with a zipper-like appearance. Because expression of langerin (CD207) confirms the presence of Birbeck granules, electron microscopy is now rarely done for diagnosis. 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. Limited skin disease often responds to topical steroids, nitrogen

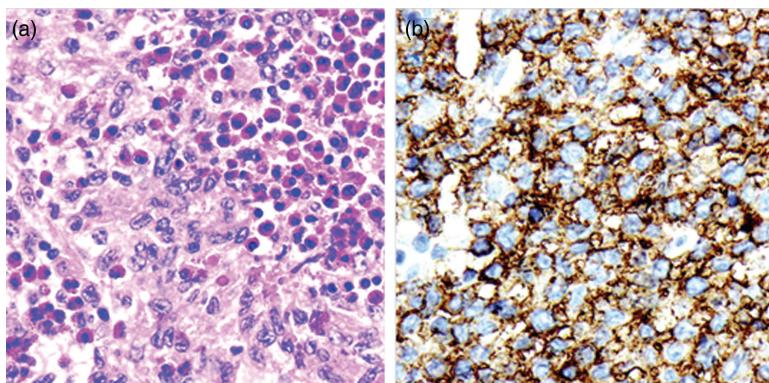


Figure 15-7 Langerhans cell histiocytosis.
(a) Hematoxylin–eosin stain demonstrating Langerhans cell infiltrate. Cells have abundant eosinophilic cytoplasm with variably shaped nuclei ranging from cleaved, grooved, folded, indented, and even lobated. Clusters of eosinophils surround the infiltrate. (b) CD1a immunohistochemistry staining Langerhans cells. From ASH Image Bank, #3461-3465.

mustard, or psoralen and ultraviolet A (PUVA) light therapy. Management of lung disease includes discontinuation of smoking; treatment with prednisone, vinblastine, 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 progressive 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.

Non-Langerhans cell histiocytoses

A number of other rare histiocytic disorders that are phenotypically distinct from Langerhans cells have been characterized. Juvenile xanthogranuloma, the most common of these disorders, is a proliferative disorder of young children that generally appears as a solitary or multiple red, yellow, or brown papular skin lesions. The condition generally follows a benign clinical course and usually resolves spontaneously, although extracutaneous lesions do rarely occur.

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. 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, relapses can occur, and the condition occasionally can be fatal.

Lysosomal storage diseases

Lysosomal storage diseases are a collection of approximately 50 genetically inherited disorders characterized by a deficiency or defect in one 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. Gaucher disease and Niemann-Pick disease are a type 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 and are of particular importance to hematologists because they frequently present with cytopenias and hepatosplenomegaly.

Gaucher disease

Clinical case

A 23-year-old male 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 an Hgb 8.0 and platelet count of $40 \times 10^9/L$ and has marked hepatosplenomegaly with a spleen measuring 12 cm below the costal margin. A BM biopsy reveals the presence of lipid-laden macrophages consistent with Gaucher cells infiltrating the marrow. Measurement of leukocyte glucocerebrosidase is reduced markedly measuring at <10% of normal levels.

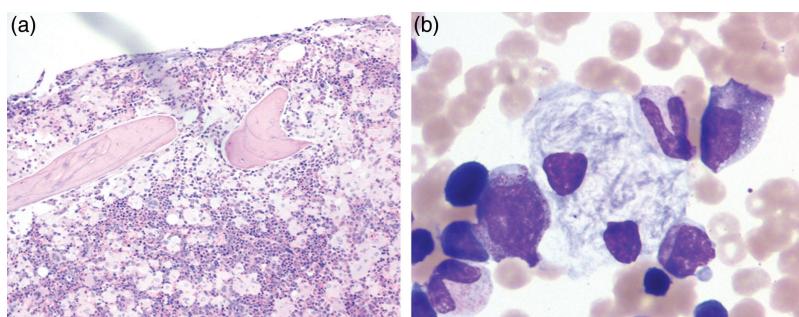


Figure 15-8 Gaucher disease. (a) Proliferation of benign-appearing macrophages with interspersed normal hematopoietic elements. (b) High-power view of bone marrow aspirate demonstrating a Gaucher cell, an abnormal macrophage with the characteristic “wrinkled-paper” cytoplasm. From ASH Image Bank, #2710-2711.

Gaucher disease is a lipid storage disease that results from the deficiency of glucocerebrosidase (acid β -glucuronidase), which hydrolyzes glucocerebroside to glucose and ceramide. Deficiency of the enzyme causes glucocerebroside accumulation in the cytoplasm of tissue macrophages, known as Gaucher cells, resulting in a characteristic wrinkled-paper appearance (Figure 15-8).

Gaucher disease is the most common lysosomal storage disease, with an incidence of approximately 1 in 75,000 births, and is more common in Ashkenazi Jewish populations, with an incidence of 1 in 1,000. Gaucher disease is inherited as an autosomal-recessive disorder, with over 300 mutations having been described. The disease is divided into three clinical subtypes based on pattern and severity of neurologic involvement. Type I (nonneuropathic) is most common (90% of all patients), has the most variable clinical presentation, and is associated with the highest residual enzyme activity. Symptoms consist of hepatosplenomegaly, cytopenias, and bone disease. Skeletal manifestations include osteopenia, pain crises, and osteolytic lesions, with radiographs showing flaring of the ends of the long bones (Erlenmeyer flask deformity) and cortical thinning. Although the clinical severity may not be predicted by the genotype, early onset is associated with more rapidly progressive and severe disease. Type II (acute neuronopathic) is rarest, with the lowest enzyme activity. Disease onset occurs during infancy and results in progressive neurologic deterioration that includes generalized seizures, hypertonia, profound mental retardation, and death during infancy. Type III (subacute neuronopathic) falls between types I and II in incidence, enzyme activity, and clinical severity. Onset occurs at any time during childhood, and manifestations include progressive dementia and ataxia, bone and visceral involvement, and supranuclear palsies.

The diagnosis of Gaucher disease can be established by enzyme assay for glucocerebrosidase activity in leukocytes, fibroblasts, or urine, which is between 0% and 30% of normal values. In addition, mutational analysis of the four most common mutations of the glucocerebrosidase gene (*N370S*, *IVS2+1G.A*, *L444P*, and *1035insG* [84G.GG]) can detect 90%-95% of the mutations associated with Gaucher disease in the Ashkenazi Jewish population and 50%-75% of the

associated mutations in the general population. The onset of symptoms ranges from 2 years of age to late adulthood, and it has been estimated that up to 60% of individuals harboring the most common *N370S* mutation never present to medical attention.

Enzyme replacement therapy (ERT) is the mainstay of treatment of individuals with nonneuropathic manifestations of Gaucher disease. Imiglucerase (Cerezyme) is a recombinant modified placental glucocerebrosidase in which the glycosylation sites of the enzyme are processed to terminate in mannose sugars to improve uptake and trafficking to the lysosomes of macrophages via the mannose receptor. In clinical studies, ERT at 30-60 U/kg administered every 2 weeks normalized cytopenias and reduced organomegaly within 6-12 months, whereas skeletal symptoms improve more slowly. Studies using low-dose ERT at 15-30 U/kg/month administered more frequently at three times a week have shown that this regimen appears to produce similar effects on cytopenias and organomegaly at significantly reduced cost. Given the high cost of ERT, published guidelines advocate treatment only for symptomatic children and adults with severe disease (eg, platelet counts <60,000/ μ L, marked splenomegaly, skeletal disease). Because glucocerebrosidase does not cross the blood-brain barrier, it has limited utility in neuropathic forms of the disease. Velaglucerase (Vpriv) and taliglucerase (Elelyso) are alternative recombinant glucocerebrosidase preparations available for ERT. An alternative therapy, miglustat (Zavesca) acts by reducing substrate accumulation in Gaucher disease by inhibiting glucosylceramide synthase, a key enzyme in glycosphingolipid synthesis. Miglustat is available for use in the United States for patients unable to receive ERT and in Europe for adult patients with mild to moderate disease. In clinical studies, miglustat decreased liver and spleen volumes by 12% and 19%, respectively, with modest improvements in hemoglobin and platelet counts.

Niemann-Pick disease

Type A and type B Niemann-Pick disease (NPD) are caused by mutations in the sphingomyelin phosphodiesterase-1

(*SMPD1*) gene, which result in deficient sphingomyelinase activity and accumulation of sphingomyelin (ceramide phosphorylcholine). Type C NPD is an unrelated defect caused by mutations of the *NPC1* and *NPC2* genes, which result in impaired cellular processing and transport of low-density lipoprotein cholesterol.

NPD is inherited as an autosomal-recessive disorder. Type A patients have <5% of normal sphingomyelinase activity, and the disease is characterized by hepatosplenomegaly, failure to thrive, and rapidly progressive neurodegeneration, with death occurring by age 2-4 years. Examination reveals cherry-red maculae in approximately half of affected infants. Type B patients have sphingomyelinase activity within 5%-10% of normal, often have minimal to no neurologic involvement, and can survive into adulthood. Cytopenias and hepatosplenomegaly are typical, and patients can develop progressive pulmonary infiltrates.

The histologic hallmark of NPD is the pathologic foam cell or Niemann-Pick cell; these cells are histiocytes filled with lipid droplets or particles that are uniform in size, giving the cells a “mulberry-like” or “honeycomb-like” appearance, and are found in involved organs. Type A and B NPD may be readily diagnosed by assays for sphingomyelinase in leukocytes or cultured fibroblasts, which demonstrate reduced activity (1%-10%) in the disease. Genetic testing can detect the most common mutations in type A, which account for approximately 90% of the mutant alleles in the Ashkenazi Jewish population. Currently, no specific treatment exists for NPD.

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.
- Gaucher disease is a lysosomal storage disorder caused by mutations in glucocerebrosidase, leading to abnormal accumulation of glucocerebroside in tissue macrophages and resulting in hepatosplenomegaly, cytopenias, and skeletal disorders. ERT can reverse both nonhematologic and hematologic manifestations.

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Myeloproliferative neoplasms
Ross L. Levine and Ramon V. Tiu

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CHAPTER
16



Myeloproliferative neoplasms

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Introduction

Myeloproliferative neoplasms (MPNs) are a phenotypically diverse group of stem cell-derived clonal disorders characterized by proliferation of one or more of the components of the myeloid lineage (ie, erythroid, granulocytic, megakaryocytic, or mast cell). In 1951, William Dameshek first used the term *myeloproliferative disorders* (MPDs) and grouped together these four similar and overlapping clinicopathologic entities (ie, chronic myelogenous leukemia [CML], primary myelofibrosis [PMF], polycythemia vera [PV], and essential thrombocythemia [ET]). In 2008, the World Health Organization (WHO) revised the classification of MPDs and renamed this group of disorders as MPNs to underscore their clonal nature (Table 16-1). The updated 2008 classification featured the following major modifications, aside from the nomenclature update: (i) mastocytosis has been included in the MPN category; (ii) diagnostic criteria for ET, PV, and PMF have been updated; and (iii) a new category of myeloid or lymphoid neoplasms with eosinophilia and

abnormalities of *platelet-derived growth factor receptor a* (PDGFRA), *platelet-derived growth factor receptor b* (PDGFRB), or *fibroblast-growth factor receptor 1* (FGFR1) was created.

From a practical standpoint, classification of MPNs is currently in transition from traditional morphologic assessments toward a disease-defining and disease-associated molecular genetic classification. Genetic studies have identified clonal genetic abnormalities involving cytoplasmic or receptor tyrosine kinases (TKs) in the majority of MPN patients. These genetic abnormalities, most commonly translocations or point mutations, result in abnormal, constitutively active TK signaling that leads to pathologic proliferation of myeloid precursors. The four major or classic MPNs are *BCR-ABL*-positive CML, PV, ET, and PMF (formerly known as chronic idiopathic myelofibrosis or agnogenic myeloid metaplasia). At present, CML is the only one of the four major MPNs characterized by a disease-defining genetic abnormality—the t(9;22)(q34;q11) Philadelphia chromosome and its molecular equivalent, the aberrant *BCR-ABL* fusion gene. Although not specific for any MPN, activating point mutations of Janus kinase 2 (JAK2) TK (JAK2 V617F) are observed in almost all patients with PV and in a significant proportion of patients with ET, PMF, and other myeloid disorders. Aside from somatic activating JAK2 exon 12 mutations (JAK2 V617F-negative PV) and myeloproliferative leukemia (MPL) mutations (in ET and PMF patients) in some JAK2 V617F-negative MPN patients, a spectrum of somatic mutations in genes involved in various cellular processes also have been identified, including in

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Off-label drug use: Dr. Levine: not applicable. Dr. Tiu: Thalidomide in anemia in myelofibrosis. Cladribine in treatment of hepatomegaly post-splenectomy in myelofibrosis. Interferon-alpha as cytoreductive therapy for polycythemia vera and essential thrombocythemia and pregnancy in essential thrombocythemia.

Table 16-1 Current classification of myeloproliferative neoplasms.

Myeloproliferative neoplasms (MPNs)
Chronic myelogenous leukemia, <i>BCR-ABL1</i> positive
Chronic neutrophilic leukemia
Polycythemia vera
Primary myelofibrosis
Essential thrombocythemia
Chronic eosinophilic leukemia, not otherwise specified
Systemic mastocytosis
MPN, unclassifiable
Myeloid (and lymphoid) neoplasms associated with eosinophilia and abnormalities of <i>PDGFRA</i> , <i>PDGFRB</i> , or <i>FGFR1</i>
Myeloid and lymphoid neoplasms associated with <i>PDGFRA</i> rearrangement
Myeloid neoplasms associated with <i>PDGFRB</i> rearrangement
Myeloid and lymphoid neoplasms associated with <i>FGFR1</i> abnormalities

genes that regulate DNA methylation (*TET2*, *DNMT3A*, *IDH1/IDH2*), histone modification (*ASXL1*, *EZH2*, *IDH1/IDH2*), RNA splicing (*SF3B1*, *U2AF1*, *ZRSR2*, *SRSF2*), signal transduction (*LNK*, *CBL*, *NRAS*), and transcription factors (*RUNX1*, *TP53*). In addition, systemic mastocytosis (SM) frequently is associated with somatic mutations in *c-KIT* (*KIT D816V*) and *TET2* and less frequently with mutations in *DNMT3A*, *CBL*, *SF3B1*, and *ASXL1*. Unclassifiable MPN refers to clonal, TK-negative syndromes with features that may be shared by specific MPNs, but they have atypical findings and fail to meet specific diagnostic criteria for specific MPNs or other related hematologic disorders. Therefore, the presence of a specific MPN-associated mutation is of diagnostic significance, but the absence of *JAK2*, *MPL*, or other MPN-associated disease alleles does not exclude a diagnosis of a specific MPN.

The different MPNs share several clinical and laboratory features, including frequent organomegaly (hepatomegaly or splenomegaly) caused by sequestration of excess blood cells or abnormal proliferation of hematopoietic cells; increased metabolic rate; hypercellularity of the bone marrow (due to clonal marrow hyperplasia) associated with increased numbers of granulocytes, red blood cells, or platelets; and absence of significant dysplasia. Despite their insidious onset, each MPN has the potential to undergo a stepwise progression that terminates in marrow failure due to ineffective hematopoiesis caused by fibrosis or transformation to blast phase, which is clinically similar to acute leukemia. By definition, MPNs must have <20% blasts.

Epidemiology

Each of the specific MPNs is relatively uncommon. The incidence for each of the major MPNs varies from 0.4–2.8

cases per 100,000 persons per year. The National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) database began collecting data on chronic myeloproliferative disorders in 2001. The cumulative age-adjusted SEER incidence rate during this period varied between 2.1 and 2.4 per 100,000 persons per year. MPNs are primarily neoplasms of adults, with a peak in frequency between the fifth and seventh decades of life. In fact, it is assumed that only 5% of the patients with PV are <40 years old at diagnosis. Adult-type MPNs, especially *BCR-ABL*-positive CML and ET, however, occur only rarely in children.

Chronic myelogenous leukemia, *BCR-ABL1* positive

Clinical case

A 60-year-old male construction worker with a history of coronary artery disease and hyperlipidemia came to see a physician in a free clinic for persistent fatigue that started about 2 months prior. He complains of intermittent episodes of palpitations, dizziness, weight loss, and discomfort over the left upper quadrant of the abdomen. Physical examination was remarkable only for palpable splenomegaly measuring 11 cm below the left subcostal margin. Routine complete blood count (CBC) showed leukocytosis (white blood cell [WBC] = $40 \times 10^9/L$) with predominance of neutrophils and neutrophil precursors, normocytic anemia (hemoglobin [Hgb] = 10.2 g/dL, hematocrit = 35%, mean corpuscular hemoglobin = 85 fL), and normal platelet counts (platelet = $335 \times 10^9/L$). Also noted on laboratory examination are basophilia (absolute basophil count = $2 \times 10^9/L$) and eosinophilia (absolute eosinophil count [AEC] = $3 \times 10^9/L$). A bone marrow biopsy was performed and showed a hypercellular marrow (90% cellularity) with granulocytic proliferation. Metaphase cytogenetics showed t(9;22) (q34;q11) [20] and fluorescent in situ hybridization (FISH) showed the *BCR-ABL1* fusion gene.

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 Philadelphia (Ph) chromosome.

Epidemiology

CML accounts for 15%–20% of leukemia cases in adults. The worldwide annual incidence of CML is 1–2 cases per 100,000 persons, with a slight male predominance (male-to-female ratio, 1.3:1). The median age at diagnosis is between 50 and 60 years. In children, *BCR-ABL1*-positive CML is most commonly seen in the 10- to 14-year-old age-group

and accounts for only 2% of childhood leukemia. Radiation exposure has been implicated as a risk factor; however, 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)] initially was identified in patients with CML in 1960. The t(9;22)(q34;q11) translocation in CML juxtaposes the 3' segment of the *c-ABL* oncogene (normally encoding the Abelson 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-ABL* mRNA, which, in turn, is translated into a functional abnormal protein. At diagnosis, 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, fourth chromosome or cryptic translocations. In these cases, routine cytogenetic analysis is unable to detect the Ph chromosome, and the diagnosis relies on demonstration of the fusion transcript by either FISH or reverse transcriptase (RT) polymerase chain reaction (PCR).

Three separate breakpoint regions in the *BCR* gene are associated with distinct disease phenotypes. In typical CML, the *BCR* gene is interrupted at either its e13 or e14 loci (exons 12-16). Collectively, this region 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 (b2a2) and e14a2 (b3a2). These transcripts are translated into 210-kd proteins, collectively known as p210BCR-ABL. Importantly, the rearranged *c-ABL* segment here includes sequences necessary for TK activity. As a result, the p210BCR-ABL oncprotein 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 survival.

Two alternative translocations involving *BCR* and *ABL* also have been implicated in the pathogenesis of hematologic malignancies (Figure 16-1). In one of these, a similar segment of *c-ABL* is transposed onto a locus of *BCR* that is downstream (39) from the M-BCR locus, a region referred to as μ-BCR (exons 17-20). Translocations involving μ-BCR yield a larger fusion gene than those involving M-BCR, and this larger fusion gives rise to a 230-kd

p230BCR-ABL protein. The p230BCR-ABL product has been found in uncommon CML variant cases that are characterized by chronic neutrophilia with or without thrombocytosis and a more indolent disease course than CML associated with the p210BCR-ABL. Distinction between p230BCR-ABL CML and chronic neutrophilic leukemia can be challenging. The third type of *BCR-ABL* rearrangement juxtaposes the same *c-ABL* segment to the minor *BCR* breakpoint region (m-BCR), which is located upstream (59) from the M-BCR (exons 1-2). The resultant smaller chimeric oncogene generated by this rearrangement gives rise to a 190-kd p190BCR-ABL protein product. The p190BCR-ABL transforming protein most often is found in a portion of de novo acute lymphoblastic leukemia (ALL) cases referred to as Ph-positive ALL. Rarely, the p190BCR-ABL product can be detected in CML, either coexpressed with p210BCR-ABL or detected alone in atypical cases that are associated with monocytosis. Coexpression of p190BCR-ABL and p210BCR-ABL 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 or molecular abnormalities is associated with progression to accelerated and blast phases of disease or resistance to TK inhibitors. At the chromosomal level, additional mutations are identified in 50%-80% of advanced-disease cases. These changes include monosomy 7, t(3;21), amplification of t(9;22), trisomy 8, trisomy 19, and abnormalities of chromosome 17. At the molecular level, mutations in the kinase domain of *BCR-ABL*, which are the most prevalent mechanism of imatinib resistance in patients with CML, have a reported annual resistance rate of <1%-7% in newly diagnosed patients in the chronic phase, with the incidence decreasing over time. To date, more than 50 different mutations have been associated with different degrees of clinical resistance to TK inhibitors.

Clinical features

Roughly 90% of patients with CML present in the chronic phase of disease, most commonly with an insidious onset, which is diagnosed based on abnormalities on a CBC. Nearly 20%-40% of individuals are asymptomatic and are discovered incidentally. Common symptoms at presentation include fatigue, night sweats, and weight loss and are normally due to hypercatabolic symptoms, splenomegaly, anemia, or platelet dysfunction. Hyperleukocytosis alone does not routinely cause symptoms because of the relative maturity of the leukemic cells compared with those seen in

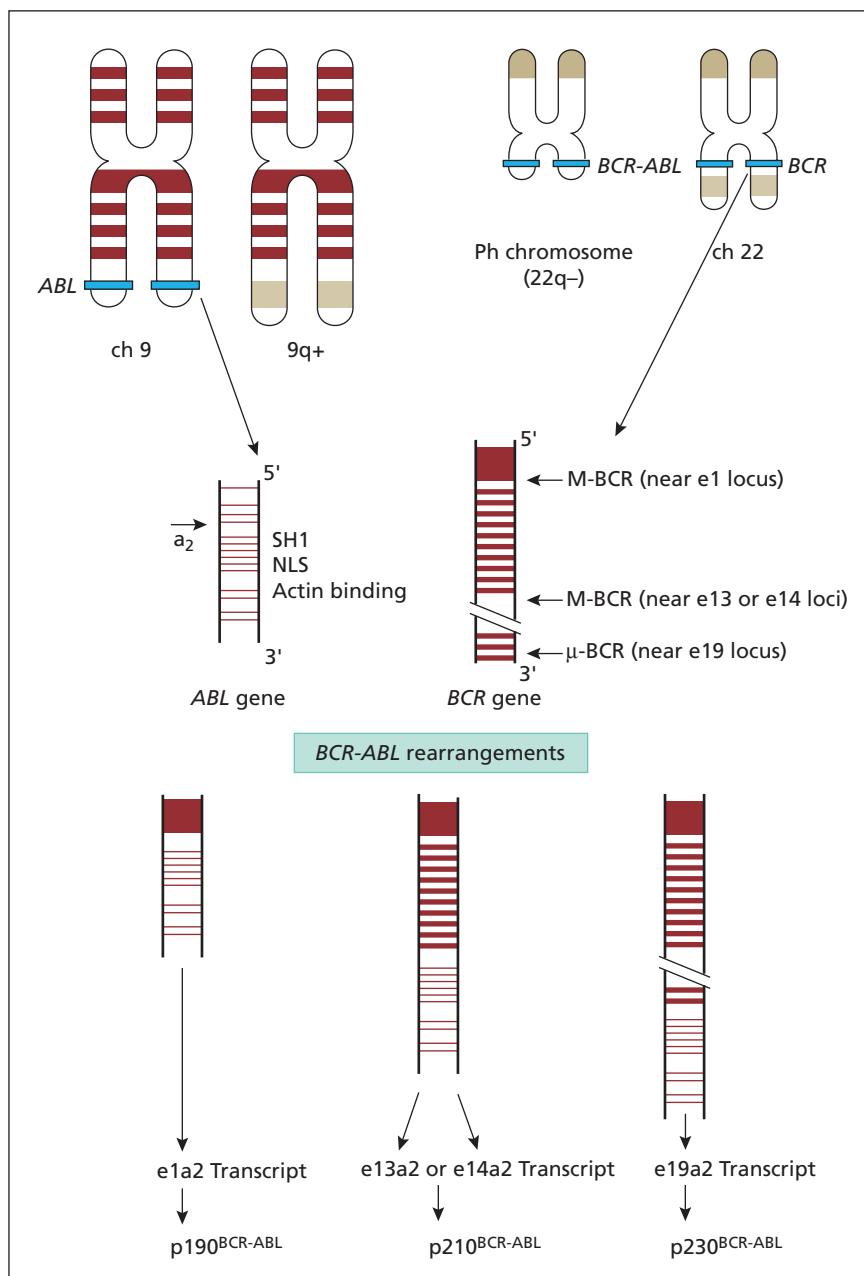


Figure 16-1 Schematic of molecular pathogenesis of t(9;22)(q34;q11) in chronic myelogenous leukemia (CML). The 39 portion of the *ABL* gene on the telomeric region of the long arm of chromosome 9 is translocated to the *BCR* gene on chromosome 22 to form the characteristic 22q- abnormality referred to as the Philadelphia (Ph) chromosome. Breakpoints within the *ABL* gene occur within introns 1b or 2, both of which are 59 (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 1 of 3 different locations within *BCR*, yielding hybrid oncogenes of varying length consisting of 59 *BCR* sequences and 39 *ABL* sequences. Each hybrid oncogene gives rise to a chimeric transcript, which encodes a fusion protein with oncogenic activity. These include p190^{BCR-ABL} (resulting from fusion at the minor breakpoint or M-BCR site), p210^{BCR-ABL} gene product (resulting from fusion at the major breakpoint or M-BCR site), and p230^{BCR-ABL} (resulting from fusion at the micro breakpoint or μ-BCR site).

acute leukemia; however, males with very high WBC counts rarely present with leukostasis-related priapism.

Most patients will present with splenomegaly (50%-90%) at diagnosis, and painless hepatomegaly may be present in up to half of the patients. Thrombotic and hemorrhagic complications are relatively infrequent (<5%), although purpura is a common complaint. Bleeding with CML becomes a major concern during the blast phase of disease.

Diagnostic criteria

The laboratory and marrow abnormalities in chronic-phase, accelerated-phase, and blast-phase CML are summarized in

Table 16-2. In the peripheral blood, neutrophilia and immature circulating myeloid cells are hallmark features of CML. More than 50% of patients present with a WBC count >100,000/mL, 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 half of patients. Roughly 15%-35% of patients present with platelet counts >700 × 10⁹/L, although extreme thrombocytosis (ie, >1,500 × 10⁹/L) is uncommon. Patients with very high platelet counts may be at greater risk of thrombotic or hemorrhagic complications. The high cell turnover and hypercatabolic state of CML are associated with elevated lactate dehydrogenase (LDH) and uric acid levels. The leukocyte

Table 16-2 Clinical features of chronic myelogenous leukemia.

Symptoms	Laboratory abnormalities	WHO classification
<i>Chronic phase</i>		
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-ABL</i> rearrangement (usually p210 <i>BCR-ABL</i>)	
Bleeding/bruising	High LDH	
Priapism	Hyperuricemia	
	Marrow myeloid and megakaryocytic hyperplasia, mild/moderate fibrosis, <10% blasts, minimal dysplasia, t(9;22) ± other abnormalities	
<i>Accelerated phase</i>		
Progressive splenomegaly and infarcts	Karyotypic evolution with increased blasts	10%-19% of WBCs in peripheral blood and/or nucleated bone marrow cells
Progressive weight loss and sweats	Blood or marrow blasts ≥10%	Peripheral blood basophils ≥20%
Unexplained fever or bone pain	Blasts and promyelocytes ≥20%	Persistent thrombocytopenia (<100 × 10 ⁹ /L) unrelated to therapy, or persistent thrombocytosis (>1,000 × 10 ⁹ /L) unresponsive to therapy
	Basophils plus eosinophils ≥20%	Increasing spleen size and increasing WBC count unresponsive to therapy
	Platelet count <100,000/mL	Cytogenetic evidence of clonal evolution
	Increasing peripheral counts or cytopenias unresponsive to antileukemic therapy	Megakaryocytic proliferation?
	Increasing marrow fibrosis	
<i>Blast phase</i>		
Bleeding, bruising	Blood or marrow blasts ≥20%	Blasts ≥20% of peripheral blood white cells or of nucleated bone marrow cells
Infections	Myeloid blast phenotype	Extramedullary blast proliferation
Prominent constitutional symptoms	Lymphoid blast phenotype	Large foci or clusters of blasts in the bone marrow biopsy
Massive splenomegaly	Biphenotypic or undifferentiated blasts	
Tissue manifestations of extramedullary disease		

LDH = lactate dehydrogenase; WBC = white blood cell count; WHO = World Health Organization.

alkaline phosphatase (LAP) score is almost always low or 0, in contrast to the high LAP scores observed in patients with other MPNs or with reactive neutrophilia. The marrow in chronic-phase CML typically shows myeloid hyperplasia and an elevated myeloid-to-erythroid ratio (often >10:1). Bone marrow blasts are usually <5%. Maturation of precursors is normal in CML, and dysplastic features are not routinely found. Marrow basophilia is noted in one-fourth of cases. Increased reticulin fibrosis is found in 30% of the cases and may have negative prognostic impact on outcomes. Pseudo-Gaucher cells and sea-blue histiocytes, secondary to increased cell turnover, frequently are found.

The quickest and least expensive way to confirm a suspected case of CML is to assay the peripheral blood for either

the *BCR-ABL* fusion gene or its chimeric transcripts. The most widely used techniques involve FISH and RT-PCR, and the sensitivity 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, 200-500 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-ABL* transcript in <1 of 10⁵ cells. 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 p210BCR-ABL product and the p190BCR-ABL product. Because of the lower cost and ability to discriminate the breakpoints, RT-PCR is the preferred molecular assay for CML diagnosis. Quantitation of *BCR-ABL*, either by FISH or RT-PCR, is not particularly helpful at the time of diagnosis but becomes important for clinical decision making and monitoring of minimal residual disease in patients on therapy. With the use of TK inhibitors for chronic-phase CML, sensitive measures of treatment response have become critical in patient management decisions. Because the false-positive rate of FISH for *BCR-ABL* fusion gene is ~3%, quantitative RT-PCR is the preferred technique for monitoring disease response. Although a positive RT-PCR or FISH assay confirms the diagnosis of CML, a complete staging of the disease still requires a marrow evaluation to rule out advanced-stage CML (Table 16-2). The marrow sample is necessary to assess the percentage of undifferentiated blasts and to evaluate for the presence of additional cytogenetic abnormalities. Conventional cytogenetic studies identify a Ph chromosome in 90%-95% of cases; more than half of the karyotypically negative cases will have a detectable *BCR-ABL* rearrangement by molecular assay. The clinical course of *BCR-ABL*-positive, Ph-negative patients is identical to that of patients with Ph-positive CML. Additional cytogenetic abnormalities usually are not found at diagnosis in patients with early stage disease.

Treatment

The development of imatinib mesylate and second-generation TK inhibitors has completely changed standard therapeutic approaches for all phases of CML. Other therapies, however, still can serve an adjunctive role or, in the case of conventional allogeneic stem cell transplantation (SCT), potentially can be curative to some patients. It is unknown whether TK inhibitor therapy will offer a subset of patients the chance for lifelong disease remission, although the majority of patients on TK therapy remain in clinical or cytogenetic remission for 5-7 years or longer.

Imatinib mesylate and other treatments aimed at achieving remission

The promise of targeted therapy for CML was realized with the approval of the first small-molecule TK inhibitor for cancer, imatinib mesylate, in May 2001. Imatinib binds the adenosine triphosphate (ATP) binding site in the catalytic domain of the *BCR-ABL* oncogene and inhibits the *BCR-ABL* 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 c-KIT TKs. These actions are useful for the treatment of other hematopoietic (eg, SM without KIT mutations, chronic eosinophilic leukemia [CEL]) and nonhematopoietic (eg, gastrointestinal stromal tumor) disorders, but they can cause minor in vivo adverse effects (eg, anemia) in patients with CML when imatinib is used at the standard 400 mg/d dose. Higher doses of imatinib (eg, 600-800 mg/d) result in higher rates of cytogenetic and molecular remission, but with a modest increase in treatment adverse effects. In phase I clinical studies of chronic-phase CML patients who were either intolerant of or resistant to interferon- α (IFN α), imatinib administered at doses >300 mg/d yielded a complete hematologic response (CHR) rate of 98%. The majority of the responses occurred by 4-6 weeks; 31% and 13% of patients achieved a major cytogenetic response (MCyR; $\leq 35\%$ *BCR-ABL*-positive metaphases) or complete cytogenetic response (CCyR; no *BCR-ABL* metaphases) at a median treatment time of 5 months. These responses were among patients who were, on average, 4 years from initial CML diagnosis. One-third of patients in this pivotal study also had features of accelerated-phase disease (other than increased blast counts). With 2-year follow-up, the majority of patients maintained a durable response on imatinib therapy, and some achieved a delayed CCyR. The U.S. Food and Drug Administration (FDA)-approved initial dose for chronic-phase disease, 400 mg/d, is very well tolerated. Infrequent adverse effects include nausea, muscle cramps, periorbital edema, diarrhea, and mild myelosuppression. In many patients who experience unacceptable adverse effects, transient dose reduction or treatment interruption allows for patients to resolve adverse effects and resume full-dose therapy.

The pivotal phase III study comparing imatinib to the combination of IFN α and cytarabine (the International Randomized Interferon and STI571 [IRIS] trial) demonstrated the superiority of imatinib compared with IFN α plus cytarabine, with higher rates of CHR, MCyR, and CCyR; freedom from progression to accelerated-phase or blast crisis CML; and better tolerance of therapy. The majority of patients who were at high risk according to current prognostic models achieved MCyR at a rate of 69%-78.9% in the imatinib arm. An 8-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. The rate of major molecular response (MMR) was 86%. None of the patients who achieved MMR at 12 months progressed to accelerated-phase or blast-phase CML. The estimated overall survival and event-free survival at 8 years were 85% and 81%, respectively. The estimated freedom from progression to accelerated- or blast-phase CML was 92%. There were low yearly rates of progression to accelerated- or blast-phase CML in years 4-8.

after starting imatinib treatment (0.9%, 0.5%, 0%, 0%, and 0.4%). Only 3% of patients who achieved CCyR progressed to accelerated- or blast-phase CML. More patients (55%) remained on imatinib therapy while the remainder have discontinued treatment due to insufficient therapeutic outcomes (16%), side effects (6%), death (3%), and other reasons (17%). 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 (600–800 mg/d) or second-generation TK inhibitors (dasatinib and nilotinib). Phase II studies demonstrate that higher dose imatinib yields higher rates of CCyR and MMRs at earlier time points for low- or intermediate-Sokal-risk newly diagnosed CML patients. A phase III clinical trial of high-Sokal-risk CML conducted by the European LeukemiaNet, however, showed no significant difference in CCyR and MMR rates at 12 months between patients treated with 400 mg/day versus 800 mg/day of imatinib. The phase III Tyrosine Kinase Inhibitor Optimization and Selectivity (TOPS) prospective randomized trial, which compared high-dose (800 mg/day of imatinib) and standard-dose imatinib (400 mg/day of imatinib), showed a higher rate of CCyR and MMR at 6 months but did not differ at 12 months. Half of the patients in the high-dose imatinib had to undergo dose reduction to less than 600 mg/day of imatinib, and there was a higher rate of grade 3 and 4 hematologic adverse events in the higher dose imatinib arm. No significant differences in CCyR or MMR were noted between the two treatment arms based on Sokal risk scores. Responses were achieved sooner in patients who received the 800 mg/day of imatinib, but longer follow-up will be necessary to determine the significance of this finding. Currently, high-dose imatinib therapy is not recommended as initial treatment for newly diagnosed chronic phase CML patients.

Second-generation TK inhibitors

Patients with CML who relapse while on imatinib therapy have been studied carefully to determine the molecular basis of imatinib resistance. In most patients, resistance is associated with reactivation of BCR-ABL TK activity and a number of possible mechanisms have been identified in tumor cell lines and patient samples, including amplification of the *BCR-ABL* gene and overexpression of the BCR-ABL protein. The acquisition of resistance in patients, however, appears primarily to be due to point mutations that affect the TK domain (TKD) of ABL, the site of imatinib binding to BCR-ABL. Kinase domain mutations occur in 50%–90% of reported imatinib-resistant cases, whereas gene amplification of *BCR-ABL* has been observed in <10% of cases.

To date, 50 different point mutations have been identified in patients with clinical resistance to imatinib. Moreover, the

risk of acquisition of imatinib resistance mutations is associated with the phase of the disease. Acquired resistance to imatinib in early and late-chronic-phase CML occurs in 15% and 25% of patients, respectively. In an analysis of 256 patients with various stages of CML, the overall incidence of TKD mutations was 26% in chronic phase, 44% in accelerated phase, 73% in myeloid blast crisis, and 81% in lymphoid blast crisis.

Dasatinib and nilotinib, were the first two second-generation TK inhibitors used for the treatment of CML. Both agents inhibit most, but not all, imatinib-resistant BCR-ABL TKDs; however, neither drug has activity against the gatekeeper T315I mutation.

Dasatinib (Sprycel; Bristol-Myers Squibb, New York, NY), a TK inhibitor with no structural similarity to imatinib with activity against Src family kinases in addition to ABL kinases, has been approved for the treatment of adults with newly diagnosed chronic-phase CML and chronic-phase, accelerated-phase, or myeloid or lymphoid blast-phase CML with resistance or intolerance to prior therapy. Dasatinib does not rely on a conformational change of ABL for binding and thus appears to be less susceptible to the development of resistant TKD mutations that alter ABL conformation. In the update of the START-C trial, patients with chronic-phase CML with resistance or intolerance to imatinib were switched to dasatinib therapy (70 mg orally twice daily), demonstrated that a CHR was attained in approximately 90% of patients (median follow-up, 15 months), and MCyRs and CCyRs were noted in 59% and 49% of patients, respectively. Although no responses were seen in patients with the T315I mutation, disease control was noted across all other TK mutations. The MMR rate at 12 months was 25%. Progression-free survival at 15 months was 90%, and overall survival was 96%. The average daily dose administered was approximately 100 mg. The efficacy of dasatinib versus imatinib in the frontline treatment of chronic phase CML patients also was investigated. The phase 3 randomized open-label trial, Dasatinib versus Imatinib study in Treatment-Naive CML-Chronic Phase (DASISION) showed that CML patients on 100 mg/d of dasatinib achieved higher CCyR (77% vs. 66%) at 12 months and MMR (46% vs. 28%) compared with patients treated with Imatinib 400 mg/d. Furthermore, the rates of accelerated- and blast-phase progression were less in the dasatinib compared with the imatinib treatment arm (1.9% vs. 3.5%). Toxicities include myelosuppression, diarrhea, fatigue, and pleural effusion. Similar response rates and decreased toxicity have been demonstrated with dasatinib 100 mg orally once daily. Data from the 2-year follow-up of patients enrolled in the DASISION trial showed that the difference in the cumulative CCyR rates between dasatinib- and imatinib-treated patients have come closer (86% vs. 82%). Dasatinib remains superior to imatinib, however, in terms of

MMR rates (64% vs. 46%) and deeper responses by 4.5 log reduction of *BCR-ABL* (17% vs. 8%). There were also fewer transformations to accelerated- or blast-phase CML in the dasatinib- versus imatinib-treated group (2.3% vs. 5%).

Nilotinib (Tasigna; Novartis Oncology, East Hanover, NJ)—a structural derivative of imatinib is a 30-fold more potent inhibitor of *BCR-ABL* activity—has been approved for the treatment of newly diagnosed chronic-phase CML and chronic-phase CML and accelerated-phase Ph-positive CML in adult patients resistant or intolerant to prior therapy, including imatinib but not blast-crisis CML. 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 accelerated- or blast-phase CML was better with the nilotinib-treated patients. Data from the 36-month follow-up showed superiority of nilotinib 300 mg or 400 mg orally twice daily compared with 400 mg orally once daily of imatinib in terms of rates of MMR defined as a ≤0.1% of *BCR-ABL*:*ABL* ratio on the international scale (IS) (73% and 70% vs. 53%), deeper molecular response 4 log reduction (MR4.0) (50% and 44% vs. 26%), rates of accelerated- or blast-phase CML progression (2 patients [0.7%] and 3 patients [1.1%] vs. 12 patients [4.2%]). The estimated 3-year overall survival are not statistically significantly different among the three groups (95%, 97%, and 94%), but the authors reported better overall survival for those treated with nilotinib compared with those treated with imatinib, if only CML-related deaths are considered (98.1% vs. 98.5% vs. 95.2%, HR = 0.35, *P* = .0356). Similarly, nilotinib has demonstrated significant clinical activity and an acceptable safety and tolerability profile in patients with imatinib-resistant or -intolerant chronic-phase CML, except in those who carry the T315I mutation.

Increasing the dose of imatinib has been demonstrated to work in the presence of some, but not all, point mutations that result in imatinib resistance or in moderate overexpression of the *BCR-ABL* protein. Many patients respond to dose escalation of imatinib, but the responses are not durable. Toxicities of imatinib therapy including cytopenias are more common at the higher dose. A recent study compared the results of switching early to dasatinib therapy versus escalating to high-dose imatinib in chronic-phase CML resistant to imatinib at daily doses from 400–600 mg. With a minimum follow-up of 2 years, all endpoints of the study (CHR, MCyR, CCyR, MMR, and progression-free survival) favored the switch to dasatinib. Thus, imatinib dose escalation should be considered only in patients unable to obtain clinical

milestones on imatinib therapy or who become intolerant to second-generation TK inhibitors.

Bosutinib, a dual Src/Abl kinase inhibitor recently was FDA approved for the treatment of adult patients with chronic-, accelerated-, or blast-phase CML who are resistant or intolerant to imatinib. The recommended dose is 500 mg orally per day. Bosutinib was approved based on a single-arm open-label multicenter study of chronic-, accelerated-, and blast-phase CML patients who received at least one prior TK inhibitor (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. The efficacy endpoint of the study is MCyR at week 24 for chronic phase CML patients and confirmed CHR and overall hematologic response (OHR) by week 48 for accelerated- or blast-phase CML patients. In chronic-phase CML patients, MCyR at week 24 was 33.8% for those who just received prior imatinib therapy and 26.9% for those who received prior imatinib followed by nilotinib or dasatinib. In the accelerated- or blast-phase CML patients, the responses included confirmed CHR at week 48 (prior imatinib = 30.4%) and OHR at week 48 (prior imatinib = 55.1%). For the blast-phase CML, 15% achieved CHR and 28.3% achieved OHR by week 48. The most common nonhematologic adverse events of any grade included nausea, diarrhea, vomiting, abdominal pain, rash, fever, and fatigue. The most common hematologic side effects of any grade included thrombocytopenia and anemia. Bosutinib currently is not FDA approved for the frontline treatment of chronic-phase CML because the phase III Bosutinib Efficacy and Safety in Newly Diagnosed Chronic Myeloid Leukemia (BELA) trial, which compared bosutinib with imatinib in newly diagnosed chronic-phase CML, did not achieve its primary endpoint rate of CCyR at 12 months. This study included 502 newly diagnosed CML patients who were randomly assigned (1:1) to bosutinib 500 mg orally once daily or imatinib 400 mg orally once daily. There was no difference in the rate of CCyR at 12 months between bosutinib compared to imatinib (70% vs. 68%, *P* = .601). The BELA trial did show that the MMR rate at 12 months was better with bosutinib versus imatinib (41% vs. 27%, *P* = .001). Bosutinib treatment also resulted in a faster time to CCyR and MMR compared with imatinib. There were also fewer on-treatment transformations to accelerated- or blast-phase CML and fewer CML-related deaths with bosutinib. Bosutinib was associated with more frequent gastrointestinal and liver-related side effects, whereas neutropenia, edema, and musculoskeletal problems were more common with imatinib.

T315I mutation results in resistance to imatinib as well as dasatinib and nilotinib because it causes a structural change that closes the ATP-binding pocket of *BCR-ABL* so that drugs cannot enter and inhibit the kinase domain. Patients

who develop resistance to imatinib, nilotinib, or dasatinib in the setting of an acquired T315I mutation are considered resistant to all three TK inhibitors and should be considered for alternative therapies, including investigational agents, allogeneic SCT, or IFN therapy depending on the patient's age, comorbidity, and donor availability. Recently, a third-generation TK inhibitor, ponatinib, has shown activity in patients with CML with T315I mutations. Ponatinib is an oral pan-BCR-ABL inhibitor with potent activity against the native enzyme and all tested resistant mutations. In the phase 2 PACE trial, refractory chronic-phase, accelerated-phase, and blast-phase CML or Ph-positive ALL resistant or intolerant to dasatinib or nilotinib, or with the resistant T315I mutation, were treated with ponatinib (45 mg orally once daily). A total of 88% of the patients in the cohort have resistance to either dasatinib or nilotinib. Chronic-phase CML patients who are resistant or intolerant to prior therapy achieved a 42% MCyR, whereas those with chronic-phase CML and T315I mutation achieved a 57% MCyR. In accelerated-phase CML patients who are resistant or intolerant to prior treatment, the MCyR is 74%. In blast-phase CML or ALL patients who are resistant or intolerant to prior therapy, the MCyR is 37%, and those with T315I mutation achieved a 27% major hematologic response.

Discontinuation of TK inhibitors

The exact duration of TK inhibitor therapy in CML patients continues to be a persistent question. Currently, most physicians would continue TK inhibitor therapy in CML patients indefinitely as long as tolerated and as long as desired responses are achieved and sustained because disease relapse can occur if imatinib therapy is discontinued. There are two ongoing studies that attempt to answer the question of whether imatinib can be discontinued safely in some CML patients. The study by Ross et al. (2008) showed that 67% of a limited cohort of CML patients ($n=18$) sustained CMR 12 months after discontinuation of imatinib therapy. The median follow-up of the study was 7 months for patients who received imatinib alone. In a prospective, multicenter, nonrandomized study called Stop Imatinib (STIM), imatinib therapy was stopped in patients who previously were treated with imatinib for >2 years, who were ≥ 18 years of age, and who achieved CMR defined as >5 log reduction in BCR-ABL and ABL levels and undetectable transcripts by quantitative RT-PCR. The median follow-up of the study was 17 months. Of the 69 patients who had at least a 12-month follow-up (median = 24 months), 39% remained in CMR, while 58% had disease relapse within 6 months after stopping imatinib therapy. All patients who had a molecular relapse, however, responded when retreated with imatinib. Prognostic factors predictive of maintenance of CMR after imatinib

discontinuation include low Sokal risk, longer duration of imatinib therapy (≥ 50 months), and male sex. The results suggests that it may be possible to stop imatinib but only in patients with sustained CMR for at least 2 years while getting imatinib and that there is a high risk of relapse usually in the first 6 months after discontinuation. Additional studies, however, may be needed to confirm these findings.

Stem cell transplantation

Allogeneic transplantation

Allogeneic SCT in CML generally is reserved for adults who fail TK inhibitors, although transplantation is still a reasonable option as first-line therapy in children and younger adults (for whom the risk-to-benefit ratio is lower). Children and younger adults with early chronic-phase CML who receive a matched sibling donor SCT have a 5-year disease-free survival rate of 60%-85%. The relapse rate among these patients is 5%-15%. Accelerated- or blast-phase CML also may be treated with allogeneic SCT for curative intent; however, survival is significantly worse because of high relapse rates and other complications related to advanced disease. Across all ages, the incidences of acute graft-versus-host disease (GVHD) range from 8%-63%, with severe and fatal GVHD affecting up to 20% and 13% of patients, respectively. Conditioning commonly involves the use of cyclophosphamide and targeted-dose busulfan. In addition, the use of peripheral blood stem cells appears to offer an advantage for advanced-stage CML, with lower relapse rates and longer disease-free survival, compared with transplantation using marrow stem cells.

Graft-versus-leukemia effect and reduced-intensity conditioning regimens

CML cells are highly susceptible to the graft-versus-leukemia (GVL) effect of an allograft. 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. The potency of the GVL effect is further illustrated by the success of donor lymphocyte infusion (DLI) for relapsed disease after SCT. DLI alone, without other therapy, induces remission in 54%-93% of patients with early hematologic or cytogenetic relapse. A therapeutic approach that relies heavily on the GVL effect against CML is allogeneic SCT following reduced-intensity or nonmyeloablative conditioning. Initial results using non-myeloablative regimens are intriguing and demonstrate

durable responses with decreased transplantation-related toxicity. The use of imatinib after reduced-intensity allogeneic transplantation also is being explored. These approaches could prove useful for patients failing nontransplantation therapies as well as those who cannot tolerate a myeloablative transplantation because of age or comorbidity.

Therapy for advanced disease

Imatinib and cytoreductive therapies

The treatment approaches for advanced CML remain unsatisfactory. Accelerated-phase CML may respond to aggressive induction-type chemotherapy regimens (25%–30% response rates) or IFN α (~40% CHR rate). Unfortunately, these responses are usually transient and are followed by rapid progression to blast crisis. Imatinib appears superior to other modalities for treatment of accelerated-phase disease. In a published phase II study, OHRs occurred in approximately 80% of patients with accelerated-phase CML (CHR, MCyR, and CCyR occurred in 53%, 24%, and 17% of patients, respectively). Overall survival and disease progression rates at 12 months were optimal among patients receiving 600 mg/d (78% and 44%, respectively). Toxicity was acceptable. Furthermore, imatinib therapy in accelerated-phase disease can serve as a bridge to SCT.

Imatinib can transiently control CML blast crisis in a proportion of patients. Both lymphoid and myeloid phenotypes respond, and optimal results are achieved with a dose of 600 mg/d, as for accelerated-phase disease. Imatinib induced OHRs in ~50% of study subjects, 8%–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 and seldom required discontinuation of therapy. The remarkable and encouraging results for blast crisis with this well-tolerated single agent have led to ongoing studies combining imatinib with conventional acute leukemia chemotherapy regimens.

The second-generation TK inhibitors (dasatinib and nilotinib) have been evaluated in patients with accelerated-phase or blast-crisis CML resistant or intolerant to imatinib. Recent studies demonstrate that second-generation TK inhibitors induce rapid and durable responses in patients with accelerated-phase CML who failed prior imatinib therapy because of intolerance or resistance, with a favorable toxicity profile. Dasatinib, at a dose of 70 mg orally twice daily, led to CHR, MCyR, and CCyR in 45%, 39%, and 32% of patients with accelerated-phase CML, respectively. Responses were

achieved irrespective of imatinib status (resistant or intolerant). The 12-month progression-free survival and overall survival rates were 66% and 82%, respectively. Nilotinib, at a dose of 400 mg orally twice daily, led to CHR, MCyR, and CCyR in 30%, 32%, and 19% of patients, respectively. The 12-month overall survival rate was 82%. Dasatinib appears to have more activity in the management of myeloid and lymphoid blast-crisis CML than nilotinib. The lack of long-term data with second-generation TK inhibitors in patients with myeloid and lymphoid blast crisis CML, however, suggests that allogeneic transplantation should be considered in patients who achieve hematologic response after second-generation TK inhibitor therapy.

Allogeneic transplantation

Although myeloablative allogeneic SCT can cure up to 40% of adult patients with CML in accelerated phase, the salvage rate of patients who receive transplantation in the setting of blast crisis is dismal. If transplantation is delayed, patients usually are treated with induction therapy or TK inhibitors to help achieve a second chronic-phase CML that may improve the success of an SCT. Transplantation in the second chronic phase yields outcomes comparable to those for transplantation in the accelerated phase (ie, 20%–40% long-term disease-free survival). With myeloid blast crisis, induction chemotherapy regimens used for acute myeloid leukemia (AML) can achieve a second chronic phase in 20%–30% of patients, whereas regimens for ALL are effective in 40%–60% of cases with lymphoid blast crisis. Aggressive chemotherapy in these settings incurs all of the complications associated with treatment of de novo acute leukemia. Responses are unstable, and a second blast crisis develops within weeks to a few months without further therapy. Moreover, recent studies suggest that the combination of induction chemotherapy and TK inhibitor therapy might increase the likelihood of response in blast crisis; however, phase II and III data are not yet available.

Course and prognosis

Chronic phase

After diagnosis, the chronic phase of CML typically remains stable for an average of 3–5 years before patients progress to accelerated- or blast-crisis CML. The rate of transformation to blast phase is 5%–10% per year during the first 2 years after diagnosis, but increases to 25% per year thereafter. Before the development of TK inhibitors, patients with CML who did not undergo SCT had a median survival of roughly 5–7 years, whereas 30% of patients survived beyond 10 years. Also before the development of TK inhibitors, multivariate prognostic models (eg, the 1984 Sokal score for patients

treated with chemotherapy, the 1998 Gratwohl score for patients considering allogeneic SCT, and the 1998 Hasford [Euro] score for IFN-treated patients) were highly useful to help predict shorter survival and make decisions regarding earlier use of aggressive modalities, such as SCT or experimental therapies. Recent studies suggest that these older prognostic models still can predict the probability of achieving a cytogenetic remission in patients treated with imatinib mesylate and second-generation TK inhibitors. In the current era of TK inhibitors, the most important prognostic indicator is the response to therapy. The three response types evaluated in patients with CML who are receiving TK inhibitor therapy are hematologic responses, cytogenetic responses, and molecular responses. Currently, the CCyR rate to front-line TK inhibitors is 70%-90%, with 5-year progression-free survival and overall survival rates between 80% and 95%. The likelihood of achieving good long-term outcomes is associated with patients achieving specific clinical milestones on imatinib therapy (Table 16-3), including the following:

- Achieving CHR at 3 months
- Achieving at least an MCyR at 12 months
- Achieving CCyR at 18 months

Recent studies have suggested that achievement of early molecular response (<10% BCR-ABL transcript level reduction at 3 months of first-line TK inhibitor therapy) is associated with durable long-term responses and survival in chronic phase CML patients. One study involving 282 patients showed that those with >9.84% BCR-ABL transcript

levels at 3 months have significantly inferior 8-year probabilities of overall survival, progression-free survival, and cumulative incidence of CMR and CCyR compared with those who have lower transcript levels. The study concluded that single measurements of BCR-ABL transcripts at 3 months is a good way to predict those patients who will do poorly. Similarly, another study reported that >10% BCR-ABL transcript level by IS at 3 months after imatinib treatment correlated with worse 5-year overall survival compared with patients with *BCR-ABL* levels of 1%-10% and <1%. The National Comprehensive Cancer Network (NCCN) 2013 guidelines currently recommend monitoring quantitative PCR every 3 months when a patient is responding to therapy. If patients do not achieve these responses, they should undergo full restaging, and if their clinical situation worsens, mutational testing and modification of therapy should be considered. In the 2013 NCCN guidelines, *BCR-ABL* kinase domain mutation testing is recommended for chronic-phase CML patients who failed to achieve PCyR or *BCR-ABL/ABL* ≤10% by IS at 3 months, CCyR at 12 and 18 months, or any loss of response (hematologic relapse, cytogenetic relapse or 1 log increase in BCR-ABL transcript levels and MMR loss).

Accelerated phase

The accelerated phase of 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

Table 16-3 Expected milestones and definition of failure and suboptimal response for previously untreated patients in chronic-phase chronic myelogenous leukemia receiving first-line imatinib mesylate therapy at a dose of 400 mg daily.

Time	Failure	Suboptimal response
Diagnosis	NA	NA
3 months after diagnosis	No hematologic response (stable disease or disease progression)	Less than CHR
6 months after diagnosis	Less than CHR, no CyR ($\text{Ph}^1 > 95\%$)	Less than PCyR ($\text{Ph}^1 > 35\%$)
12 months after diagnosis	Less than PCyR ($\text{Ph}^1 > 35\%$)	Less than CCyR
18 months after diagnosis	Less than CCyR	Less than MMR
Anytime	Loss of CHR*, loss of CCyR†, mutation	ACA in Ph^1 cells‡, loss of MMR§, mutation

Failure implies that the patient should be moved to other treatments whenever available. Suboptimal response implies that the patient may still have a substantial benefit from continuing imatinib treatment but that the long-term outcome is not likely to be optimal so patients become eligible to other therapies.

*To be confirmed on two occasions unless associated with progression to accelerated phase/blast crisis.

†To be confirmed on two occasions, unless associated with CHR loss or progression to accelerated phase or blast crisis.

‡High level of insensitivity to imatinib.

§To be confirmed on two occasions, unless associated with CHR or CCyR loss.

||Low level of insensitivity to imatinib.

ACA = additional chromosome abnormalities; CCyR = complete cytogenetic response; CyR = cytogenetic response; CHR = complete hematologic response; MMR = major molecular responses; NA = not applicable; PCyR = partial cytogenetic response; Ph = Philadelphia chromosome.

Adapted from Baccarani M, Saglio G, Goldman J, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood*. 2006;108:1809-1820.

precursors and undifferentiated blasts in the marrow, blood, and extramedullary tissue. The clinical symptoms associated with the accelerated phase (Table 16-2) may be minor, delayed, or completely absent. The median survival from the onset of accelerated phase, without an SCT or TK inhibitors, is only 12–18 months. Death occurs predominantly because of transformation to blast phase with the associated life-threatening complications of marked leukocytosis and complete failure of normal hematopoiesis. Various laboratory criteria have been proposed for defining entry into accelerated phase, some of which have been identified by multivariate analyses as prognostically useful. Patients with karyotypic evolution alone, except for the acquisition of chromosome 17 abnormalities, without accompanying clinical and other laboratory changes, may follow a more indolent course to blast transformation.

Blast-phase CML (leukemic progression)

Progression of CML to acute leukemia, synonymous with “blast phase” and “blast crisis,” evolves most commonly from a preceding accelerated phase and is reached when the proportion of blasts in the blood or marrow is ≥20% (Table 16-2). Blast crisis may develop suddenly, without an intervening accelerated phase, in up to one-fourth of chronic-phase patients. By comparison, a sudden transformation from chronic phase to blast phase occurs in >5% of patients on IFN α therapy. Sudden transformation to blast phase in patients taking imatinib is observed with an annual rate of 1%–2%. Myeloid lineage markers (eg, CD33, CD13, CD14, and CD15) are expressed by the blast cells in more than half of the cases of blast-phase CML. Up to one-third express B-cell–precursor lymphoid markers (eg, CD10, CD19, and CD20). Undifferentiated acute leukemia and cases displaying both myeloid and lymphoid cell surface markers account for the remainder. Most CML cases express the p210BCR-ABL gene product, and only rare cases are associated with p190BCR-ABL alone. Thus, a case of Ph-positive ALL that subsequently is found to be associated with p210BCR-ABL might actually represent CML presenting in lymphoid blast crisis. The clinical and laboratory features of blast-phase CML are summarized in Table 16-2. Cytogenetic abnormalities in addition to t(9;22) are found in 65%–80% of cases. The overall median survival is 3–6 months for older patients and roughly 8 months for younger adults; patients with lymphoid blast crisis survive 4–5 months longer than those with myeloid blasts. In blast phase, 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 end-organ extramedullary leukemic infiltration.

Chronic neutrophilic leukemia

Clinical case

A 64-year-old previously healthy female 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: WBC = $27 \times 10^9/L$, Hgb = 12.9 g/dL, hematocrit = 40%, mean corpuscular volume (MCV) = 88 fL, platelet = $315 \times 10^9/L$, absolute neutrophil count (ANC) = $25 \times 10^9/L$, occasional metamyelocytes and myelocytes were noted but accounting for 5% of WBCs, no myeloblasts were seen. She decided to see a hematologist who noted hepatosplenomegaly and mild cervical lymphadenopathy by physical examination. A bone marrow aspiration and biopsy was subsequently performed, which showed increased numbers of neutrophilic granulocytes, hypercellular marrow (95%), no dysplastic changes, and 3% myeloblasts. Metaphase cytogenetics showed 46,XX [20]. Molecular testing for BCR-ABL, PDGFRA, PDGRB, FGR1, and JAK2 V617F were all unremarkable.

Chronic neutrophilic leukemia (CNL) is a rare chronic MPN that only recently has been recognized as a distinct entity within the 2008 WHO classification. CNL is a diagnosis of exclusion because reactive neutrophilia and other MPNs need to be excluded.

Epidemiology

CNL is an extremely rare disorder with 200 cases described to date. It appears to occur more commonly in older patients, although cases in adolescents have been described. Males and females also appear to be equally affected.

Pathobiology

No known genetic lesions have been uniquely associated with CNL. A few patients with CNL may present with heterozygous or homozygous JAK2 V617F mutations.

Clinical features

Splenomegaly is the most frequently found clinical feature in patients with CNL, often accompanied by hepatomegaly. Some patients will present with gastrointestinal tract bleeding, thrombocytopenia, pruritus, and gout. Transformation to acute leukemia has been reported.

Diagnostic criteria

CNL is characterized by sustained, mature neutrophilic leukocytosis with few or no circulating immature

granulocytes, monocytosis, or basophilia. The WBC count usually exceeds 25,000/mL. Granulocyte dysplasia is not detectable, but the granules may be coarse (toxic). Bone marrow biopsy demonstrates hypercellularity with a striking neutrophil proliferation with myeloid-to-erythroid ratio reaching up to 20:1. Blasts or promyelocytes are not increased in number in the beginning, and dysplasia and reticulin fibrosis are not evident. Cytogenetic studies are normal in ~90% of cases of CNL, but in the remaining cases, clonal karyotypic anomalies may include +8, +9, +21, del(20q), del(11q), and del(12p). No Ph chromosome or *BCR-ABL1* fusion gene is found. Furthermore, CNL should be differentiated from neutrophilic CML, which is a rare variant of *BCR-ABL* (e19/a2 junction)–driven Ph-positive CML.

Treatment

Optimal treatment for patients with CNL remains to be defined. Splenectomy has resulted in worsening of neutrophilic leukocytosis and cannot be recommended. Treatment of CNL to date has consisted largely of cytoreductive agents, such as hydroxyurea, where clinical responses are noted in 75% of cases. Median duration of responses is ~12 months. Similar to other chronic MPNs, IFN α has been used successfully and may produce durable responses. Allogeneic sibling SCT usually is reserved for patients with accelerated or blastic transformation. Given the potential for blastic transformation and progressive refractory neutrophilia, however, allogeneic SCT may be appropriate for younger patients.

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. Blastic transformation occurs 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 and AML. 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 ($n = 1$).

Systemic mastocytosis

Clinical case

A 67-year-old female nurse has been experiencing fever, chills, diarrhea, a recurrent urticarial-like rash, flushing, and palpitations for the past 5 months. She decided to see a primary care doctor who noticed palpable lymphadenopathies in the neck and axillary regions and a palpable spleen tip by physical examination. Routine blood work showed some normocytic anemia (Hgb = 10.1 g/dL, MCV = 92 fL); leukocytosis (WBC = $25 \times 10^9/L$) with increased lymphocytes (40%), monocytes (28%), and eosinophils (12%); and mild thrombocytopenia (platelets = $97 \times 10^9/L$). Review of prior blood works dating back to 6 and 8 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 spindle shaped mast cell infiltration grade of 50%. The bone marrow cellularity is 90%. 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 is 450 ng/mL. The patient was diagnosed with SM with associated hematologic non–mast cell disease, specifically SM with chronic myelomonocytic leukemia.

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. Mastocytosis encompasses a heterogeneous spectrum of disorders characterized by clonal, neoplastic proliferation of mast cells accompanied by inappropriate tissue infiltration (Figure 16-2). Clinical manifestations of mastocytic disorders are caused by uncontrolled proliferation of tissue mast cells and the release of mast cell–derived mediators. Given that SM has a spectrum of clinicopathologic features in common with MPNs, the revised 2008 WHO classification included SM under the broader umbrella of MPNs. Mastocytosis can be classified according to site and extent of involvement of mast cells as well as the biologic behavior of these cells (Table 16-4).

Epidemiology

The incidence of mastocytic disorders is poorly defined; SM is felt to be a very rare disease. Although mastocytosis can be diagnosed at any age, cutaneous mastocytosis (CM) is more common in children, whereas SM occurs predominantly in adults. These disorders appear to have a slight male predominance.

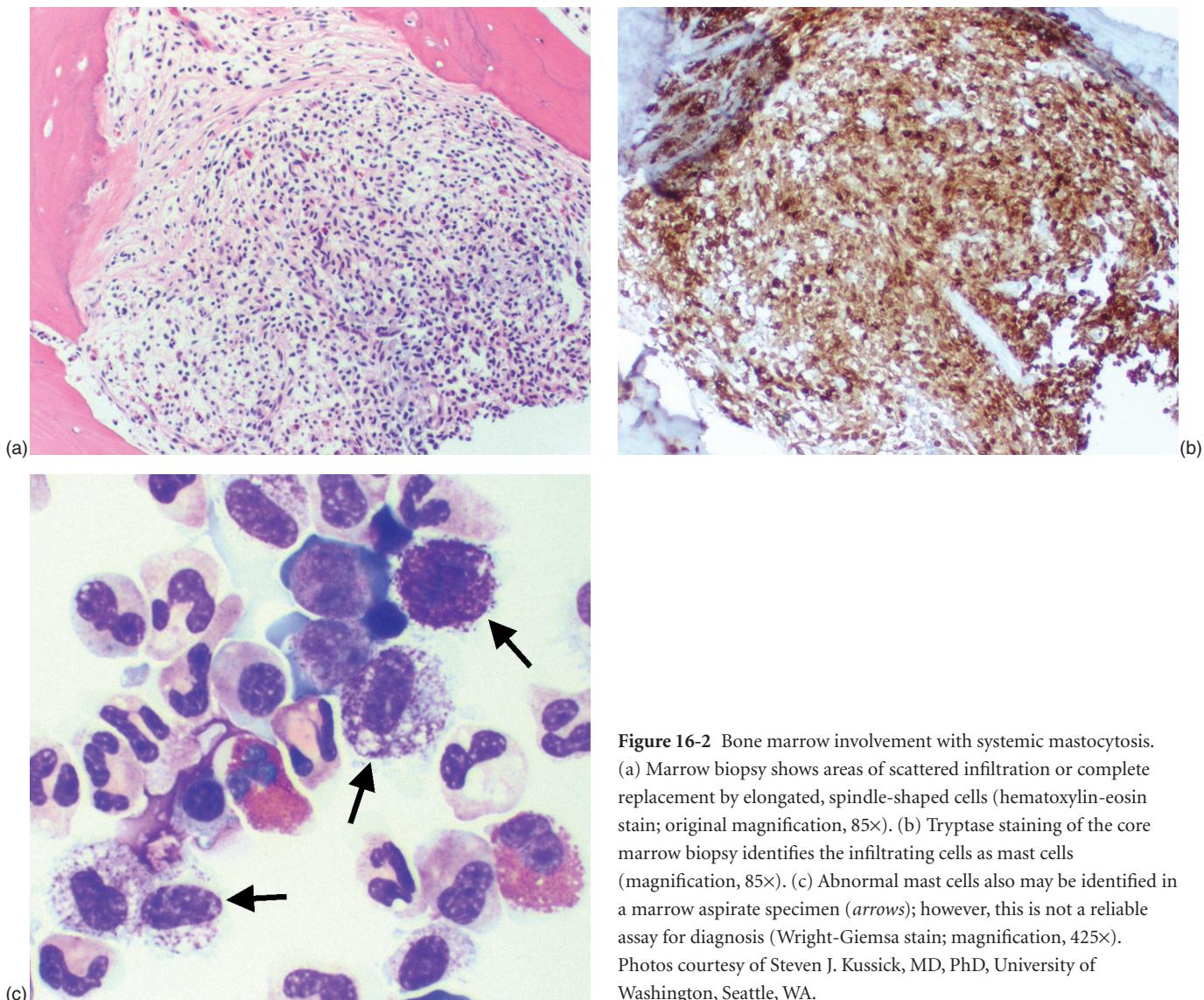


Figure 16-2 Bone marrow involvement with systemic mastocytosis. (a) Marrow biopsy shows areas of scattered infiltration or complete replacement by elongated, spindle-shaped cells (hematoxylin-eosin stain; original magnification, 85×). (b) Tryptase staining of the core marrow biopsy identifies the infiltrating cells as mast cells (magnification, 85×). (c) Abnormal mast cells also may be identified in a marrow aspirate specimen (arrows); however, this is not a reliable assay for diagnosis (Wright-Giemsa stain; magnification, 425×). Photos courtesy of Steven J. Kussick, MD, PhD, University of Washington, Seattle, WA.

Table 16-4 The 2008 World Health Organization classification of mastocytosis.

Cutaneous mastocytosis

- Urticaria pigmentosa/maculopapular cutaneous mastocytosis
- Diffuse cutaneous mastocytosis
- Solitary mastocytoma of skin

Systemic mastocytosis

- Indolent systemic mastocytosis
 - Bone marrow mastocytosis
 - Smoldering systemic mastocytosis
- Aggressive systemic mastocytosis
- Systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease (SM-AHNMD)
- Mast cell leukemia
- Mast cell sarcoma
- Extracutaneous mastocytoma

Adapted from Horny H-P, Metcalfe DD, Bennett JM, et al. Mastocytosis. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008.

Pathobiology

Most cases of mastocytosis are associated with somatic-activating point mutations of the *c-KIT*, the protein TK receptor for stem cell factor. The most common point mutations result from a Val for Asp substitution at codon 816 (D816V), which is found in ~80% of SM patients and results in ligand-independent activation of KIT. 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 variants of *KIT* point mutations also have been described. Mutations in *KIT* D816V has been identified in both the mast cell and AHNMD compartment, which potentially may indicate a shared pathogenetic origin in a hematopoietic progenitor. Recently, *TET2*, *DNMT3A*, *ASXL1*, *SF3B1*, and *CBL* mutations also have been identified in a subset of mastocytosis patients. The presence of a sole *TET2* mutation or *TET2* mutation in addition to other molecular abnormalities seem

to confer worse survival, which is supported further by the recent data showing increased proliferation and survival in the absence of cytokines of TET2-deficient mast cells. SM can be diagnosed in conjunction with another hematologic neoplasm in ~30%-40% of cases. The associated disease can either be myeloid or rarely lymphoid. Chromosomal changes detected by metaphase cytogenetics occasionally may be in patients with SM, which usually is observed in the context of a concomitant non-mast cell hematologic neoplasm. Furthermore, novel cytogenetic lesions, including uniparental disomy recently have been identified using single-nucleotide polymorphism array (SNP-A) karyotyping.

Clinical features

The presenting clinical features for patients with mastocytosis depend on the extent of disease. Approximately 80% of patients with mastocytosis have evidence of cutaneous involvement. In SM, which represents 15%-20% of mastocytosis cases, bone marrow involvement is necessary for the diagnosis, so bone marrow examinations should be performed in all patients with evidence of mast cell disease at any site. Fifty percent of SM patients present with skin involvement. Other organs commonly involved include the liver, spleen, lymph nodes, and gastrointestinal mucosa.

Cutaneous manifestations of mastocytosis typically include a reddish-brown maculopapular eruption (urticaria pigmentosa) or, less often, a diffuse erythema, plaques, nodules, or the classic description of urticaria following stroking of the skin (Darier sign). Blistering can occur in pediatric patients and represents an aggressive form of urticaria pigmentosa. Clinical features of SM are categorized in four distinct groups: (i) constitutional symptoms (eg, fatigue, fever, weight loss), (ii) cutaneous manifestations, (iii) systemic mediator-related symptoms (eg, abdominal pain, flushing, headache, hypotension), and (iv) musculoskeletal complaints (eg, bone pain and myalgias, osteopenia, fractures). SM may be indolent or more aggressive clinically. Serum tryptase levels usually are elevated in SM and represent a minor diagnostic criterion. Other clinicopathologic features important in the diagnosis of SM include B and C findings (Table 16-5).

Diagnostic criteria

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 16-5.

Treatment

Treatment of CM includes H1 and H2 antihistamines, cromolyn and other mast cell stabilizers, topical or intralesional glucocorticoids, and psoralen and ultraviolet A (PUVA) phototherapy. Adults with chronic CM may require long-term continuous or intermittent symptomatic treatment. For adult patients with indolent variants of SM, symptomatic treatment with combinations of H1 and H2 antihistamines, anticholinergic drugs, proton pump inhibitors, cromolyn and other mast cell stabilizers, or PUVA is sufficient to alleviate symptoms in the majority of patients. Some patients, however, are refractory to these supportive treatments. Patients with SM always should have at least two doses of epinephrine (epipen) 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. A major goal in the management of mastocytosis is the avoidance of known and possible inciting factors. Opioid analgesics, such as morphine and codeine, are known mast cell degranulators and may produce severe adverse reactions in sensitive individuals.

Symptomatic SM in the presence of a non-mast cell clonal hematologic disease 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 like aggressive systemic mastocytosis (ASM) coexist with low-grade myeloid neoplasms, the aggressive SM may take precedence. 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. IFN α can be helpful for patients with painful skeletal lesions or mast cell tumors that threaten bony integrity. Corticosteroids generally are avoided in this case because of their potential adverse effects on bone density. Patients with evidence of end-organ damage without major bony complications should receive a combination of corticosteroids and IFN α . Roughly one-half of patients will respond to this regimen, although most responses are only partial. Single-agent cladribine or 2-chlorodeoxyadenosine (2-CdA), given at 5 mg/m²/day or 0.13-0.17 mg/kg/day as a 5-day treatment cycles every 4-6 weeks, induced clinical and laboratory responses (ie, decreased serum tryptase and urinary histamine metabolites) in patients with symptomatic SM. Patients who fail to respond to these interventions and those who progress to mast cell leukemia should receive multiagent antileukemic chemotherapy. Allogeneic SCT should be considered for younger patients with aggressive SM who achieve a remission with chemotherapy.

Table 16-5 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.

Major criterion

Multifocal, dense infiltrates of mast cells (≥ 15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organ(s).

Minor criteria

- 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 c-Kit 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 *PDGFRA*.

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.

“C” findings

1. Bone marrow dysfunction manifested by one or more cytopenia (ANC $<1 \times 10^9/L$, Hgb <10 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.

AML = acute myeloid leukemia; ANC = absolute neutrophil count; Hgb = hemoglobin; MDS = myelodysplastic syndrome; MPN = myeloproliferative neoplasm; SM = systemic mastocytosis; WHO = World Health Organization.

Adapted from Horny H-P, Metcalfe DD, Bennett JM, et al. Mastocytosis. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008.

The crucial role of *c-KIT* in normal mast cell development and the evidence that *c-KIT* mutations may be important in SM pathogenesis prompted treatment of mastocytosis patients with TK inhibitors. Imatinib mesylate, because of its inhibitory properties against KIT, was the first to enter the clinical arena. The presence of *KIT* D816V mutation confers resistance to imatinib mesylate by affecting the catalytic pocket of the *c-KIT* protein, preventing imatinib from binding and exerting its inhibitory activity. Therefore, *KIT* mutation analysis is important in therapeutic decision making in SM. Nonetheless, a trial of imatinib should be considered in patients with aggressive SM who lack the D816V mutation. Other TK inhibitors also appear to have promising effects in patients with *KIT*-D816V-positive SM. PKC412 (midostaurin) is a novel TK inhibitor that has displayed potent activity (inhibitory concentration at 50% of 30–40 nM) against *KIT*-816 mutants. On the basis of these data, in one study, PKC412 100 mg twice a day was administered to patients with aggressive SM in continuous 28-day cycles until progression or intolerable toxicity. In a phase II study, 26 patients with SM were treated and resulted in a 69% response rate (major response rate of 38%, good partial response rate of 19%, and a minor partial response of 12%). Major response was defined as normalization of 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. Toxicities encountered with the use of PKC412 include nausea, vomiting, fatigue, and diarrhea. Nilotinib, dasatinib, and MLN518 appear to have a similar preclinical cytotoxic profile against *KIT*-D816V-positive mast cells, but their clinical efficacy appears to be limited in this disease type.

Course and prognosis

Life expectancy can be quite variable, ranging from only a few months in aggressive SM to 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. Predictor factors of poor prognosis in SM include late onset of symptoms, absence of CM, low platelets, hypoalbuminemia, hepatosplenomegaly, anemia, and elevated LDH. Cytoreductive therapy should be considered for patients with aggressive variants of SM. Recent data also suggest that the presence of sole *TET2* mutations or *TET2* in combination with *DNMT3A* and *ASXL1* mutations may predict for worse survival in SM patients.

Chronic eosinophilic leukemia, not otherwise specified

Clinical case

A 35-year-old male graduate student came to the university health clinic because of non-productive cough, diarrhea, fatigue, intermittent fevers (102°F), and muscle aches. He attributed it to stress for an upcoming finals examination but was wondering why this has been going on for 2 months now. He has never been sick with any illnesses. The clinic doctor had him get a CBC, and it showed the following: WBC = $19 \times 10^9/L$, Hgb = 11.5 g/dL, MCV = 83 fl, platelets = $188 \times 10^9/L$, ANC = $12 \times 10^9/L$, and AEC = $3.4 \times 10^9/L$. There were 3% circulating blasts in the peripheral blood. Workup for connective tissue diseases, parasitic infections, allergies were unremarkable. He was asked to see a hematologist or oncologist, who he saw 3 weeks later. CBC from this visit again showed leukocytosis with elevated eosinophil counts. He had a bone marrow aspiration and biopsy, which showed 6% bone marrow blasts with no dysplastic changes. Metaphase cytogenetics showed 46, XY [20]. He has no abnormalities in *PDGFRA*, *PDGFRB*, *FGFR1*, *BCR-ABL*, and *INV 16*.

CEL is a newly defined entity of MPNs characterized by an autonomous, clonal proliferation of eosinophil precursors resulting in persistent elevation of eosinophils in the peripheral blood, bone marrow, and peripheral tissues. By definition, in CEL, not otherwise specified (CEL-NOS), the absolute peripheral blood eosinophil count has to exceed $1.5 \times 10^9/L$, and patients must not have the Ph chromosome (*BCR-ABL1* fusion gene) or rearrangements of *PDGFRA*/ *PDGFRB* or *FGFR1*. End-organ damage can be a manifestation of direct eosinophil leukemic infiltrate or secondary to the release of cytokines or other enzymes by release of their toxic granules. CEL-NOS requires demonstration of eosinophil clonality and must be differentiated from idiopathic hypereosinophilic syndrome (HES). Idiopathic HES is defined as a persistent (>6 months) peripheral blood eosinophilia ($1.5 \times 10^9/L$) without a clear underlying cause and without evidence of eosinophil clonality but associated with end-organ damage or dysfunction.

Epidemiology

Although CEL-NOS is a rare MPN, the true incidence of these neoplasms is unknown. Complicating matters further, the revised 2008 WHO classification now separates previously classified patients with CEL into myeloid (and lymphoid) neoplasms associated with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1* or CEL-NOS depending on the presence or absence of rearrangements of *PDGFRA*/*PDGFRB* or *FGFR1*, respectively. Nonetheless, eosinophilic syndromes seem to occur much

more often in men than in women, with a male-to-female ratio of approximately 9:1. The peak incidence is in the fourth decade, but CEL-NOS can occur at any age, including childhood. The true incidence of idiopathic HES will remain unclear until further defined.

Clinical features

Approximately 10% of cases of CEL-NOS are identified incidentally. More commonly, patients present with complaints of symptoms such as fever, fatigue, cough, pruritus, diarrhea, angioedema, and muscle pain. Most patients with idiopathic hypereosinophilia will develop signs of end-organ damage within 3 years of diagnosis. Clinically, CEL-NOS can manifest with a plethora of symptoms. The most serious clinical findings relate to endomyocardial fibrosis due to eosinophilic infiltration of the heart, leading to constrictive pericarditis, fibroplastic 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, papulonodular lesions, and erythematous plaques. Gastrointestinal involvement by eosinophilia can result in ascites, diarrhea, gastritis, colitis, pancreatitis, cholangitis, or hepatitis.

Diagnostic criteria

The WHO criteria for diagnosis of CEL-NOS exclude 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. The revised 2008 WHO criteria also require the presence of eosinophilia ($1.5 \times 10^9/L$); presence of 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*, or *FGFR1* rearrangements; bone marrow blasts $<20\%$; and the absence of inv(16)(p13.1q22). Idiopathic HES is best classified in patients who have the following characteristics: (i) persistent eosinophilia ($\geq 1.5 \times 10^9/L$) lasting for at least 6 months; (ii) no reactive causes of eosinophilia; (iii) no associated clonal myeloid neoplasm like AML, MDS, MDS/MPN, MPN, and SM; (iv) no cytokine-producing immunophenotypically aberrant T-cell population; (v) no increased myeloblasts in the peripheral blood or bone marrow; and (vi) 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. Lymphocyte variant hypersinophilia also needs to be excluded.

Treatment

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. In the past, corticosteroids (prednisone 1 mg/kg/d) have 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 hydroxyurea. Lack of steroid responsiveness warrants consideration of cytotoxic chemotherapeutic agents, such as vincristine and etoposide; these agents also have been used in patients with HES resistant to other therapies and in patients with aggressive CEL. IFN α can elicit sustained hematologic and cytogenetic remissions in idiopathic HES and CEL-NOS patients refractory to other therapies, including prednisone and hydroxyurea. Anti-interleukin (IL) 5 antibody approaches (eg, mepolizumab) have been undertaken in HES based on the cytokine's role as a differentiation, activation, and survival factor for eosinophils. Mepolizumab inhibits binding of IL-5 to the α -chain of the IL-5 receptor expressed on eosinophils. It has not yet achieved approval by the FDA for CEL-NOS but is available on a compassionate use protocol. Treatment with anti-IL-5 monoclonal antibodies could elicit rapid reductions in the peripheral blood eosinophil count (<48 hours) or could decrease serum levels of eosinophil mediators. The effective use of alemtuzumab (anti-CD52 monoclonal antibody) in refractory HES based on the expression of the CD52 antigen on eosinophils has been reported in two separate case reports. A study that included 11 patients with HES and CEL used alemtuzumab in escalating doses of 5, 10, 30 mg intravenously from days 1-3, 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. Despite these results, the data on alemtuzumab remains limited and the drug is best considered an investigational therapy for this condition at this time.

Course and prognosis

CEL-NOS and idiopathic HES have a variable course and overall survival, although both tend to be chronic disorders.

Blast transformation can occur, usually many years after diagnosis. Poor prognostic features include marked splenomegaly, cytogenetic abnormalities, and dysplastic myeloid features in the bone marrow. In one series, including patients with idiopathic HES and eosinophilic leukemia, 80% of patients were alive at 5 years from diagnosis, and 42% were alive at 15 years. Thus, close follow-up and judicious use of treatment interventions can lead to long survival.

Polycythemia vera

Clinical case

A 60-year-old male violinist came to the local clinic because of intractable generalized pruritus. The general practitioner immediately noticed multiple skin excoriations. He was prescribed antihistamines and steroid cream and was sent home. A week later, the patient came back anxious complaining of the same problem. He told the doctor that he also started to experience facial flushing and painful erythematous swelling of all his fingers. Physical exam revealed erythematous swelling of both hands, multiple skin excoriations, and palpable spleen 15 cm below the left subcostal margin. Vital signs including oxygen saturation were within normal limits. The doctor ordered a CBC, which showed the following: WBC = $12 \times 10^9/\text{L}$, Hgb = 19 g/dL, mean corpuscular hemoglobin: 85 fl, platelet count = $530 \times 10^9/\text{L}$. Additional blood tests showed a serum erythropoietin level of <2 U/L and the presence of a JAK2 V617F mutation. The patient was started on aspirin 81 mg by mouth once daily with improvement in pruritus and also erythematous swelling of both hands. She has no history of bleeding or blood clots.

Polycythemia vera (PV) is defined by an elevated red cell mass (RCM) in the absence of conditions that induce secondary erythrocytosis, such as hypoxia or inappropriate erythropoietin (EPO) production. Excessive red blood cell production by the bone marrow in PV often is associated with concomitant increases in circulating platelets and granulocytes because the acquired defect underlying PV involves a multipotential hematopoietic progenitor cell. 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. There is a slight male predominance. The median age at diagnosis is 60–65 years, with roughly 5% of cases occurring in those <40 years old. Radiation exposure, but not exposure to other environmental or toxic factors, has been linked to the disease.

Pathobiology

Clonality

PV was among the first hematopoietic disorders to be demonstrated to have a clonal origin; studies of blood cells from

women with PV who were germline heterozygous for glucose-6-phosphate dehydrogenase (G6PD) isoenzyme types expressed either the normal G6PD type or the variant type, but not a balanced mix of normal- and variant-type cells, as would be expected in a polyclonal cell population. More recently, molecular analyses of other polymorphic X-linked genes have yielded similar findings. Moreover, circulating red blood cells, platelets, granulocytes, and sometimes B-lymphocytes in patients with PV are all progeny of the malignant clone. By comparison, the majority of lymphocytes and natural killer cells are usually polyclonal and do not exhibit the karyotypic changes or other genetic abnormalities that characterize the abnormal clone. Despite this, clonal analysis using JAK2 mutational analysis frequently identifies a subpopulation of JAK2-mutant hematopoietic cells in all hematopoiesis subtypes, including lymphocytes, demonstrating that PV and other JAK2-mutant MPNs arise in the pluripotent hematopoietic stem cell compartment.

JAK2 mutations

Mutational studies have shown that a somatic-activating mutation at codon 617 in the JAK2 TK are observed in 90%–95% of patients with PV. JAK2 is an intracellular signaling molecule that is coupled to several cell surface hematopoietic growth factor receptors that lack intrinsic kinase domains, including the EPO receptor and the thrombopoietin (TPO) receptor. The specific JAK2 point mutation most closely associated with MPN, V617F, causes constitutive activation of the JAK2 kinase domain, which results in erythropoiesis losing its dependence on EPO signaling and becoming virtually autonomous. JAK2 V617F can be found in >90% of patients with PV, and diagnostic mutation assays are now available for routine clinical testing in numerous reference laboratories. This mutation is homozygous in at least one-third of PV cases, a situation that arises by 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 in most, but not all, JAK2 V617F-negative PV patients. 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 erythrocytosis without associated thrombocytosis or leukocytosis. Despite the high frequency of JAK2 V617F mutation in PV, this molecular mutation is not specific to this disease type but also is frequent in other MPN like ET and PMF. This raises the question of what other factors contribute to the phenotypic heterogeneity within different MPNs that share the same JAK2 V617F mutation. Several factors have been implicated, including differences in

allele burden, downstream intracellular signaling, host genetic background, acquisition of other molecular mutations or changes, and the hematopoietic progenitor tissue type targeted by the *JAK2* V617F mutation. A germline haplotype (46/1, GGCC) at the 3' region of *JAK2* also has been associated with a three- to fourfold increased risk of developing a *JAK2* V617F mutant or *MPL* mutant MPN, although a weak association with *JAK2* V617F wild-type MPN patient also was observed.

Other biologic abnormalities

Before the discovery of *JAK2* V617F, a number of physiologic, growth, and survival abnormalities were identified in progenitor cells from PV patients. One such characteristic biologic feature, which has been used as a minor diagnostic criterion for PV by several groups, is the ability of erythroid progenitors to form colonies in serum-containing cultures in the absence of EPO, a phenomenon often called endogenous erythroid colony (EEC) growth. EEC growth is not specific for PV; it also can be observed in some cases of ET and PMF and in rare cases of congenital polycythemia. *JAK2* V617F provides a mechanistic explanation for this phenomenon in acquired MPNs. Additional phenotypic features of unclear pathogenic significance include decreased expression of c-Mpl (the TPO receptor) in PV megakaryocytes and platelets and overexpression of messenger RNA (mRNA) encoding polycythemia rubra vera 1 (*PRV1*) in mature granulocytes but not in myeloid progenitors. Neither of these findings is specific for PV, although some groups use them as supportive evidence for a PV diagnosis in challenging cases. More recently, somatic loss-of-function mutations in the putative tumor suppressor genes *TET2* and *ASXL1* have been identified in a subset of PV, ET, and PMF patients. Although these mutations contribute to the pathogenesis of these MPNs, they also frequently are observed in myelodysplastic syndrome (MDS) and AML patients; thus, they are not useful from a diagnostic perspective. Recent data, however, suggest that the presence of *ASXL1* mutation may be associated with an increased propensity for PV and ET to transform to a myelofibrotic state.

Qualitative platelet defects and inappropriate granulocyte activation can occur in PV. Platelets may contain abnormally low levels of serotonin and adenine. In vitro aggregation responses to epinephrine or collagen may be incomplete or absent. Acquired type 2 von Willebrand disease (vWD) may occur with extreme thrombocytosis (usually $>1,000 \times 10^9/L$) and can contribute to clinical bleeding. The mechanism of acquired vWD in thrombocytosis appears to be related to platelet binding, cleavage, and ultimately clearance of large von Willebrand factor (vWF) multimers. Normal vWF activity is restored after the platelet count is normalized by

cytoreductive drugs or plateletpheresis. Granulocyte activation may induce endothelial cell injury and plasma coagulation activation, contributing to thrombosis risk.

Clinical features

At presentation, approximately 80% of patients with PV are symptomatic; the rest are discovered incidentally as a result of a blood count performed for another reason. Up to one-half of patients with PV complain of headache, pruritus (particularly after bathing in hot water), and fatigue. One-third suffers from dyspnea, dizziness, visual changes, weight loss, epigastric pain, excessive sweating, or painful paresthesias of the hands and feet (erythromelalgia) (Table 16-6). These symptoms are believed to relate to hyperviscosity, hypercatabolism, elevated histamine release, and microvascular or vasomotor instability. Roughly 15% of patients diagnosed with PV will have suffered an arterial or, less commonly, a venous thrombotic event within the previous 2 years. One-fifth of patients with PV present with a large-vessel thrombotic complication, such as a transient ischemic attack, cerebrovascular accident, myocardial infarction, deep venous thrombosis, or hepatic vein thrombosis. It is important to consider the diagnosis of PV in people who develop an apparently unprovoked thromboembolic event, in particular at unusual sites (eg, dural sinuses or mesenteric veins). In particular, a substantial proportion of patients who present with Budd-Chiari syndrome are found to be *JAK2* V617F positive and have EEC formation and clinicopathologic features of PV not previously diagnosed. Epistaxis is

Table 16-6 Classical features of polycythemia vera.

Symptoms*

Fatigue, headache, pruritus, weakness, dyspnea, dizziness, visual change, weight loss, epigastric pain, excessive sweating, paresthesias (erythromelalgia), symptoms related to large-vessel thrombosis (arterial or venous), epistaxis, gastrointestinal or other mucocutaneous bleeding

Clinical signs*

Systemic hypertension, splenomegaly, plethora, hepatomegaly, cutaneous ulcers, gouty features, pulmonary hypertension

Additional laboratory abnormalities

JAK2 V617F mutation, elevated LAP score, elevated B12 level, elevated LDH, elevated uric acid, EEC growth, *PRV1* granulocyte overexpression, decreased *c-Mpl* expression, marrow hypercellularity with megakaryocyte clustering

* More common → less common.

EEC = endogenous erythroid colony; LAP = leukocyte alkaline phosphatase; LDH = lactate dehydrogenase.

Updated and modified from Berlin NI. Polycythemia vera: diagnosis and treatment in 2002. *Expert Rev Anticancer Ther.* 2002;2:330-336.

reported by 15%-20% of patients at diagnosis, and gastrointestinal bleeding is reported by approximately 5%.

Clinical findings at PV diagnosis include splenomegaly (50%-80%), facial or conjunctival plethora (~60%), hypertension (~50%), hepatomegaly (~50%), and, less commonly, cutaneous ulcers or gouty features (Table 16-6). Pulmonary hypertension has been noted in some patients with PV, ET, or PMF. Myeloid progenitor cells can be seen in the lungs of these patients on bronchoscopic biopsy, and these cells elute vasoactive cytokines that may contribute to blood vessel damage and fibrosis. The median survival after developing pulmonary hypertension is only 18 months.

Differential diagnosis

Absolute polycythemia versus relative polycythemia

An elevated hematocrit may result from either an increase in the total RCM (absolute polycythemia) or a decrease in the total plasma volume (relative polycythemia). The latter condition usually is due to moderate to severe intravascular dehydration, such as that due to diarrhea or loss of fluid into third spaces (effusions or edema), sometimes exacerbated by diuretic use. Because laboratory normal ranges are based on statistical distributions, 2.5% of healthy people will have a hematocrit value above the normal laboratory reference range. In contrast, in some cases, a normal hemoglobin and hematocrit can be a sign of disease; examination of old blood counts may demonstrate that the patient's hemoglobin has increased substantially from his or her personal baseline, although the level is still within the laboratory normal range. In addition, patients with fluid overload can present with a normal hematocrit in the setting of an elevated RCM; this is most common in patients with Budd-Chiari syndrome and resultant liver dysfunction, which masks erythrocytosis and can confound the diagnosis of PV.

Formerly, determination of RCM by isotope testing was performed routinely to evaluate an elevated hematocrit, with the goal of differentiating relative polycythemia from absolute polycythemia. In a nuclear medicine RCM assay, an aliquot of the patient's red blood cells is collected and labeled with a chromium-51 radiotracer and then reinfused into the patient. Radiolabeled albumin (iodine-125-albumin) is also injected to allow assessment of the plasma volume. Blood then is withdrawn after the radioisotopes have had time to dilute in the circulation, and photon emission from the radioisotopes is measured with a calibrated gamma counter. Absolute polycythemia is diagnosed when the total red blood cell volume is calculated to be $>36 \text{ mL/kg}$ in men or $>32 \text{ mL/kg}$ in women. The precise role of RCM testing in PV diagnosis has changed subsequent to the discovery of JAK2 V617F; the revised WHO diagnostic criteria do not require

demonstration of an elevated RCM in patients who present with an elevated hemoglobin ($>18.5 \text{ g/dL}$ in men or $>16.5 \text{ g/dL}$ in women) and are positive for JAK2 V617F. Hemoglobin values $>18.5 \text{ g/dL}$ in men of European or Asian descent, $>17.5 \text{ g/dL}$ in African men, and $>16.5 \text{ g/dL}$ in women usually do not require formal RCM determination because these values almost always reflect a true elevation in RCM. Such levels of hemoglobin elevation generally cannot be achieved by conditions associated with plasma volume depletion alone, except in the most extreme circumstances, which should be clinically obvious. Diagnostic thresholds for hematocrit values also have been defined, with values $>60\%$ in men and $>56\%$ in women consistently correlating with absolute elevations in RCM. There are also some patients, who have an elevated platelet count or other MPN-associated features (eg, splenomegaly or unprovoked thrombosis in an unusual site) and a normal hemoglobin and hematocrit, in whom RCM testing will disclose an unsuspected elevated erythroid burden. Finally, iron deficiency can result in a normal RCM in a patient with true PV, and microcytic indices in a nonthalassemic patient with MPN features should suggest this possibility. The revised WHO diagnostic criteria for PV are summarized in Table 16-7.

Primary erythrocytosis versus secondary erythrocytosis

In an adult with a marked elevation of his or her hemoglobin or elevated RCM, additional studies are required to differentiate PV from secondary causes of erythrocytosis (Table 16-8). Rare patients may have both PV and a secondary cause of erythrocytosis. Most acquired secondary polycythemic states are associated with elevated or high-normal serum EPO levels, which may be appropriately elevated (eg, in the setting of chronic tissue hypoxemia) or inappropriately elevated (eg, due to exogenous administration of recombinant EPO or endogenous EPO overproduction by the kidney or liver or by a tumor). A smoking history (including exposure to secondhand smoke) should be obtained; arterial blood gas and carboxyhemoglobin determinations should be obtained in smokers or those with occupational exposure to hydrocarbon fumes. An oxygen saturation $<92\%$ suggests the possibility of EPO-driven polycythemia. Evidence for underlying lung or cardiac disease should be sought, and evaluation for sleep apnea through monitoring of nocturnal oxygen saturations should be considered. An abdominal ultrasound is useful to assess for renal or hepatic cysts or tumors or for splenomegaly, if it is not obvious from physical examination. In the absence of other causes of elevated EPO and with a normal oxygen saturation, an oxygen dissociation curve (partial pressure of oxygen at which hemoglobin is half saturated) should be determined to evaluate for a high oxygen-affinity hemoglobinopathy, if there is sufficient clinical

Table 16-7 Diagnostic criteria for polycythemia vera.

Disease	Polycythemia vera
Major	<ol style="list-style-type: none"> 1. Hemoglobin >18.5 g/dL in men, >16.5 g/dL in women, or other evidence of increased red blood cell volume* 2. Presence of <i>JAK2</i> V617F or other functionally similar mutation such as <i>JAK2</i> exon 12 mutation
Minor	<ol style="list-style-type: none"> 1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation 2. Serum erythropoietin level below the reference range for normal 3. Endogenous erythroid colony formation in vitro
Diagnosis	Requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria

* Hemoglobin or hematocrit .99th percentile of method-specific reference range for age, sex, altitude of residence or hemoglobin >17 g/dL in men, >15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL from an individual's baseline value that cannot be attributed to correction of iron deficiency, or elevated red blood cell mass >25% above mean normal predicted value.

Adapted from Swerdlow SH, Campo E, Harris NL, et al., eds. *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008.

suspicion. The family history is often suggestive in these cases. Alternative causes of secondary polycythemia also must be considered, including EPO-producing renal or liver tumors; in addition, cerebellar hemangiomas, uterine myomas, ovarian tumors, parotid tumors, lymphomas, and adrenal tumors can be associated with pathologic EPO production. Budd-Chiari syndrome can be a presenting sign of PV; however, in the acute setting, hepatic necrosis can be associated with transiently elevated EPO levels, misleadingly suggesting a secondary, EPO-driven erythrocytosis. Therefore, it is important to assess patients after they recover from the acute complications of Budd-Chiari syndrome. Androgen replacement therapy or androgen abuse by aspiring athletes and bodybuilders may induce polycythemia. Absolute polycythemia may occur in up to 15% of patients after renal transplantation. This complication usually develops 8-24 months after transplantation and remits spontaneously in one-fourth of patients; when treatment is required, therapy with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers often is effective. *JAK2* V617F mutations have not been identified in patients with secondary polycythemia without a concurrent diagnosis of a MPN; therefore, the presence of the *JAK2* V617F allele supports a diagnosis of PV even in patients with concomitant secondary polycythemia.

Differentiation between PV and secondary polycythemia is important both for prognosis and treatment because secondary polycythemia does not carry a risk of leukemic or fibrotic transformation and has a lower risk of thrombosis. In either case, however, symptoms may result from increased viscosity of the blood. In secondary polycythemia, phlebotomy may occasionally be indicated to decrease blood viscosity and improve oxygenation when symptoms occur or prophylactically, especially when hematocrit values exceed 60%. With reduction in blood viscosity, symptoms such as

headaches, plethora, and dizziness quickly abate. Caution must be exercised, however, when the cause of secondary polycythemia is physiologically appropriate, such as in cyanotic congenital heart disease, chronic hypoxia, or high-affinity hemoglobins. Tissue hypoxemia may be worsened by phlebotomy when the physiologically appropriate compensatory increase in hematocrit is reversed. If underlying severe cardiac disease exists, 0.9% normal saline should be given concurrently with phlebotomy to compensate for the blood volume that is removed.

Familial polycytemic states

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 in the center of the European part 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 is distinct from the autosomal-dominant *VHL* mutations associated with the 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

Table 16-8 Causes of secondary polycythemia.

Neonatal
Normal intrauterine environment (Hgb F)
Twin–twin transfusion syndrome or maternal–fetal bleeds
Infants of diabetic mothers
Intrauterine growth retardation
Adrenal hyperplasia
Thyrotoxicosis
Congenital
Trisomies of 13, 18, or 21
Mutant high oxygen-affinity hemoglobin
Congenital low 2,3-bisphosphoglycerate
Autonomous high-EPO production (including Chuval-type polycythemia associated with <i>VHL</i> mutations)
Autosomal dominant polycythemia (including truncating EPO receptor mutations)
Other congenital polycythemic states
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
<i>Renal lesions</i>
Renal tumors
Renal cysts
Diffuse parenchymal disease
Hydronephrosis
Wilms tumor
Renal artery stenosis
Renal transplantation
<i>Miscellaneous tumors</i>
Parotid tumors
Cerebellar hemangiomas
Lymphomas
Uterine myomata
Cutaneous leiomyomata
Bronchial carcinoma
Ovarian tumors
Adrenal tumors
Meningiomas
Pheochromocytomas
<i>Drugs and chemicals</i>
Androgens
Epoetin- α or darbepoetin- α
Novel erythropoietic agents
Nickel
Cobalt
Hepatic lesions
Hepatomas
Cirrhosis
Hepatitis

EPO = erythropoietin; Hgb = hemoglobin.

Modified from Pearson TC, Messineo M. Idiopathic erythrocytosis, diagnosis and clinical management. *Pathol Biol (Paris)*. 2001;49:170-177.

upregulates EPO expression. More recent studies of families with autosomal-dominant heritable erythrocytosis have identified germline 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 germline 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.

Diagnostic criteria and molecular testing

In the absence of causes for secondary polycythemia and a family history of erythrocytosis and in the presence of a low serum EPO level, PV is the most likely diagnosis. The Polycythemia Vera Study Group (PVSG) originally established criteria in the 1970s to standardize the diagnosis of PV to follow a uniform group of patients on treatment protocols. In light of the discovery of *JAK2* V617F and other MPN-associated molecular lesions, the WHO has published a set of diagnostic criteria that includes genetic testing for *JAK2* V617F as a major criterion (Table 16-7). *JAK2* V617F mutation testing by PCR-based assays is >90% sensitive for PV and appears to be 100% specific for clonal myeloproliferation. *JAK2* testing is useful as a first-intention diagnostic test for evaluation of an elevated hematocrit, before any other investigation, but it cannot distinguish inapparent PV from other clonal myeloid disorders. Therefore, *JAK2* V617F mutations are not specific for PV given their occurrence in ET, PMF, and other myeloid malignancies; however, the presence of the *JAK2* V617F allele and erythrocytosis does provide two major criteria for the diagnosis of PV using the new WHO criteria. For patients who are *JAK2* V617F negative, testing for *JAK2* exon 12 is of value; however, given the wide mutational spectra of *JAK2* exon 12 mutations in *JAK2* V617F-negative PV, the absence of a *JAK2* V617F or exon 12 mutation does not exclude a diagnosis of PV.

Ambiguous cases

Some patients present with an elevated RCM and a normal or near-normal serum EPO level in the absence of other diagnostic criteria for PV and without an apparent cause for secondary polycythemia. Typical features of PV will develop in 10%-40% of such patients over time, but most cases will resolve spontaneously, or a cause of secondary polycythemia ultimately will become clear. *JAK2* testing is helpful to distinguish these groups. Some younger patients have been described with chronic “idiopathic erythrocytosis” that persists for many years without evolution to PV or development of thrombohemorrhagic complications. The etiology of this

syndrome is unclear; however, a subset of patients with idiopathic erythrocytosis who do not meet the criteria for a diagnosis of PV are *JAK2* exon 12 mutation positive.

Laboratory and histopathologic features

A low-serum or inappropriately normal-serum EPO level is common with PV. 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. Normal EPO levels also may be seen in hypoxemia-induced secondary polycythemia after the elevated RCM has restored adequate renal oxygen delivery. Elevated EPO levels strongly suggest hypoxemia or another form of secondary erythrocytosis but rarely may be seen in PV if there is also a concomitant secondary polycythemic state. Assays for EEC growth, although not readily available to most clinicians, are minor criteria for the diagnosis of PV and are helpful in the evaluation of *JAK2* V617F-negative patients who present with polycythemia. Platelets show abnormal in vitro aggregation in up to 80% of cases. Additional laboratory abnormalities that are present in at least half of PV patients include an elevated LAP score, elevated LDH, and hyperuricemia (Table 16-6).

Bone marrow evaluation is not critical for the confirmation of suspected PV when other diagnostic criteria are present, including *JAK2* V617F, but is useful in supporting the diagnosis, in establishing prognosis, or in the diagnostic evaluation of *JAK2* V617F-negative patients. Common marrow findings with PV include erythroid and megakaryocytic hyperplasia, with abnormal megakaryocytes (typically large cells with hyperlobated nuclei) organized in clusters (Figure 16-3). Advanced reticulin fibrosis (ie, grades 3 or 4) is found in <5% of cases at diagnosis, but in at least 20% after 10-15 years and >50% after 20 years of disease. Karyotypic analysis may offer diagnostic utility in ambiguous cases that are *JAK2* wild type. At diagnosis, ~15% of karyotypes from PV patients contain nonrandom chromosomal abnormalities, including trisomy 8, trisomy 9, del(13q), and del(20q). Monosomy 7, del(5q), and other abnormalities seen with AML and myelodysplasia are not common in PV. The frequency of a clonal karyotype in late-stage (>10 years) PV increases to 80%. Of note, there is no clear association between specific karyotypic abnormalities and progression to either AML or the preterminal stage of high-grade marrow fibrosis.

Course and prognosis

PV is a chronic disease that is incurable with current therapies other than SCT, which is reserved for high-risk, young patients in whom the risks of allogeneic SCT are acceptable.

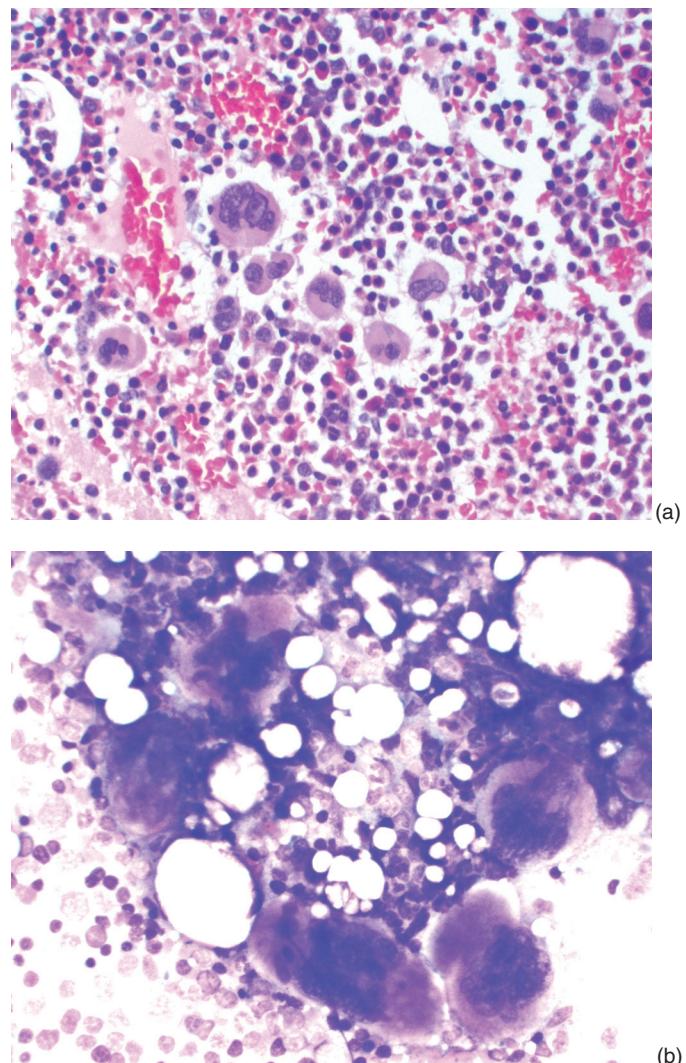


Figure 16-3 Megakaryocyte morphologic abnormalities observed in myeloproliferative disorders. (a) Megakaryocyte hyperplasia, clustering, and nuclear hyperlobation in a bone marrow core biopsy sample from a patient with polycythemia vera (hematoxylin-eosin stain; original magnification, 170 \times); (b) a marrow aspirate from a patient with essential thrombocythemia (Wright-Giemsa stain; magnification, 170 \times). Photos courtesy of Steven J. Kussick, MD, PhD, University of Washington, Seattle, WA.

Although early studies suggested that untreated erythrocytosis and thrombocytosis lead to a median survival of only 18 months because of a high incidence of fatal thromboembolic events, more recent observational studies, including younger individuals with PV and patients with chronic idiopathic erythrocytosis suggest that the clinical diagnosis of PV often is preceded by an asymptomatic prodrome of many years. Once the diagnosis is secure, treatment with phlebotomy, with aspirin, and with or without cytoreductive agents results in a near-normal life span for the average patient who is diagnosed at a median of 60-65 years of age. Although it exceeds 10 years, however, the median survival of individuals

diagnosed with PV before 40 years of age is considerably shorter than the life expectancy for a healthy person of similar age, even with active management.

Disease progression and leukemic transformation

Although PV patients commonly present with splenomegaly, marrow hyperplasia, erythrocytosis, and thrombocytosis, over time, a significant proportion of patients progress to develop progressive marrow fibrosis and compromised hematopoiesis, and then progress further to an end-stage condition often referred to as postpolycythemic myelofibrosis. Postpolycythemic myelofibrosis is characterized by progressive hepatosplenomegaly due to extramedullary hematopoiesis, advanced marrow fibrosis, and pancytopenia with leukoerythroblastosis. The median survival time in patients with postpolycythemic myelofibrosis is <3 years. At least 25%-50% of patients with postpolycythemic myelofibrosis develop AML, although not all PV patients who transform to AML will progress through a postpolycythemic myelofibrosis phase. The presence or absence of splenomegaly and the degree of leukocytosis or thrombocytosis have not been useful predictors of the disease course. In addition, current studies do not clearly demonstrate that *JAK2* mutation status or allele burden predicts survival or transformation rate in a clinically meaningful manner, although the *JAK2* V617F allele burden does increase over time in most patients.

Transformation to AML occurs in roughly 1%-3% of patients treated with phlebotomy alone. Phosphorus-32 (³²P) treatment, chlorambucil, busulfan, and alkylating agent combinations are associated with increased risk of transformation to AML (up to 15-fold increased risk in randomized PVSG trials). The European Collaboration on Low-dose Aspirin in Polycythemia Vera (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. Leukemias that occur after chemotherapy or radiation rarely may be associated with abnormalities of chromosome 5 or 7. Although early observational studies suggested that AML transformation might be increased in patients receiving hydroxyurea, the largest prospective PV study to date, the ECLAP study, enrolled 1,638 patients and noted no increase in AML in patients treated with hydroxyurea, with median a follow-up time of 8.4 years after PV diagnosis and 2.5 years after study enrollment. There is no evidence that IFN α or anagrelide are leukemogenic.

Thrombohemorrhagic risk

Thrombosis and bleeding are the major causes of morbidity and death with PV. Clinical risk factors for thrombosis and

bleeding are listed in Table 16-9. Data from the 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); these 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. The etiology of hypercoagulability in PV is not well understood, but hyperviscosity due to uncontrolled erythrocytosis (at hematocrit >50%-55%), qualitative platelet dysfunction, and granulocyte activation likely contribute to the pathogenesis of thrombosis in PV. Recent studies in patients treated with aspirin and hydroxyurea suggest that WBC count is an important predictor of thrombotic risk in patients with controlled erythrocytosis, suggesting that granulocyte activation contributes to thrombosis in patients treated with standard therapies. It is likely that patients with known genetic hypercoagulable states and concomitant PV are at higher risk of thrombosis, although this has not been confirmed in epidemiologic studies. The risk of postoperative thrombosis or bleeding is increased in patients with PV. Up to 80% of patients with PV who undergo surgery in the context of a hematocrit or platelet count markedly above the normal range will suffer a thrombohemorrhagic event. By contrast, only 5%-10% of patients suffer these perioperative complications when their counts are normalized for at least several weeks preoperatively. Active treatment—including phlebotomy, antiplatelet therapy, and cytoreductive agents—decreases the risk of thrombosis and improves the disease course. The benefit of phlebotomy clearly was demonstrated by the PVSG trials. Randomized clinical trial data from the ECLAP study

Table 16-9 Risk factors for clinical complications of polycythemia vera.

Thrombosis

- Age >60 years old
- History of previous thrombosis (venous or arterial)
- High rate of phlebotomy

Hemorrhage

- Postoperative state with uncontrolled hematocrit or platelet count
- Platelet antiaggregating therapy (particularly in elderly)
- Thrombocytopenia during late-stage PPMM

Acute leukemia

- Previous therapy with chlorambucil, busulfan, or ³²P
- Evolution to late-stage PPMM
- Possibly pipobroman use

PPMM = postpolycythemic myeloid metaplasia.

Multiple sources.

revealed that aspirin at a dose of 100 mg/d is safe and effective at reducing the rate of vascular events among PV patients without increasing the risk of bleeding, even in patients who are at low or unclear baseline risk for such events.

Therapy

Phlebotomy

The mainstay of treatment of PV remains phlebotomy to maintain the hematocrit closer to a normal physiologic range. Generally accepted goals include a hematocrit of <45% for men, <42% for women, and <37% for pregnant women late in gestation; however, there are no randomized trial data to support these specific guidelines. Additional cytoreductive or antithrombotic therapies are used as indicated for specific conditions and risk states (Table 16-10; Figure 16-4). In younger adults, weekly or even twice weekly phlebotomy may be required at the time of initial diagnosis to control presenting symptoms rapidly. Once the hematocrit is within the desired range, the interval between

Table 16-10 Current treatment of polycythemia vera.

Risk categories	Treatment
Low	Low-dose aspirin + phlebotomy
Intermediate	Low-dose aspirin* + phlebotomy
High	Low-dose aspirin + phlebotomy + hydroxyurea

Risk stratification of PV according to thrombotic risk. High risk: age ≥ 60 years or previous thrombosis; intermediate risk: age < 60 years and no previous thrombosis, but with either platelet count $> 1,500 \times 10^9/L$ or cardiovascular risk factors (tobacco use, diabetes mellitus, hypertension, hyperlipidemia); low risk: absence of any of the aforementioned risk factors.

* Clinically significant acquired von Willebrand disease should be excluded before the use of aspirin in patients with platelet count $> 1,000 \times 10^9/L$. From Vanucchi AM, Guglielmelli P, Tefferi A. Advances in understanding and management of myeloproliferative neoplasms. CA Cancer J Clin. 2009;59:171-191.

phlebotomies may be extended to 3-6 months. Aggressive initial phlebotomy without adjunctive myelosuppressive therapy may be associated with a higher risk of thrombosis, particularly in the elderly and those with a prior history of

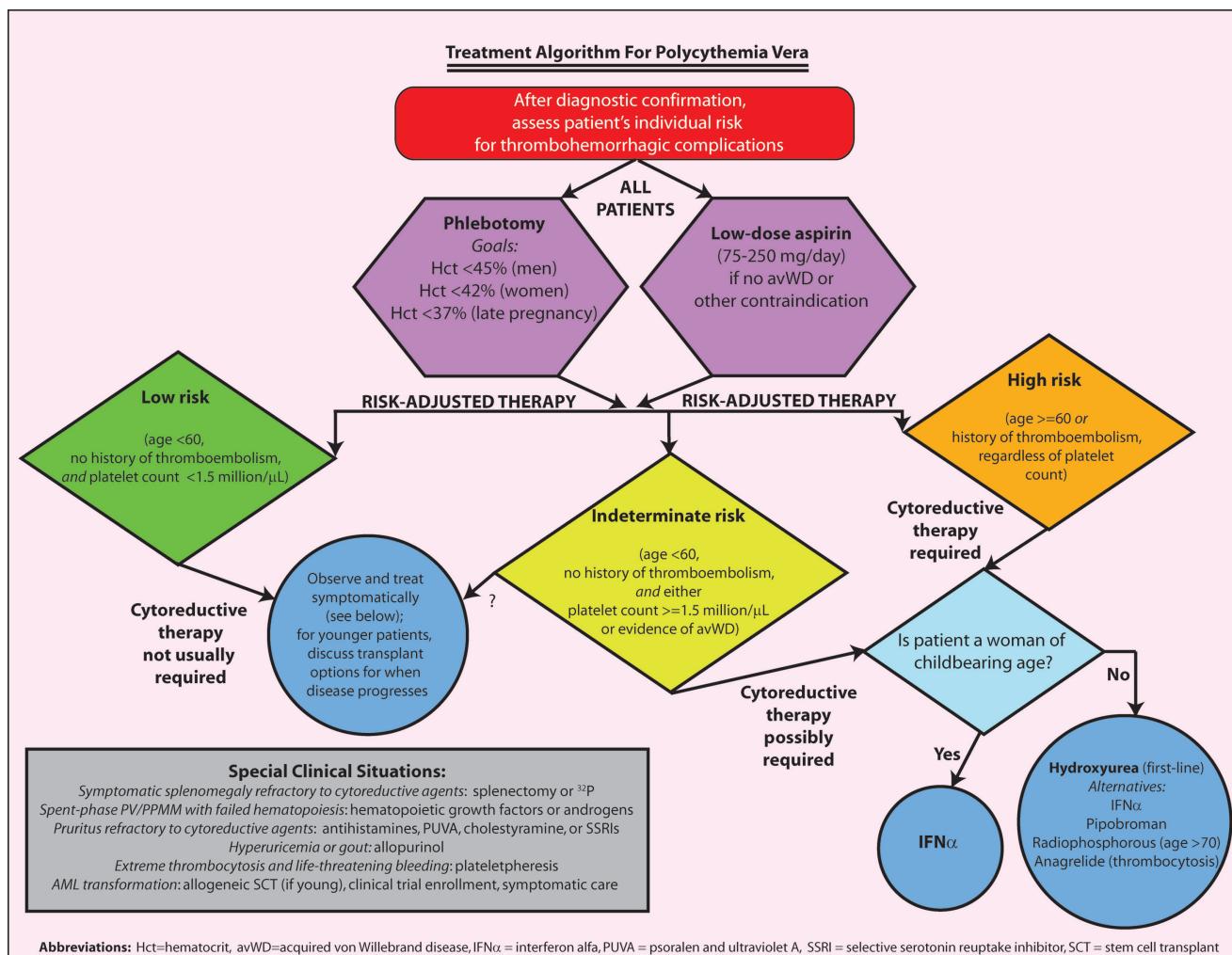


Figure 16-4 A suggested treatment algorithm for polycythemia vera. Assessment of the patient's thrombohemorrhagic risk is the key step.

thrombosis; this has been hypothesized to be the result of new platelet formation in response to the thrombopoietic stimulus of rapid phlebotomy. On the basis of these clinical observations, myelosuppressive therapy is recommended as part of the initial treatment of patients >60 years of age and younger patients with thrombotic risk factors, particularly a history of thrombosis. Phlebotomy to the point of iron deficiency without concomitant cytoreductive therapy may be associated with reactive elevation in the platelet count, although the thrombohemorrhagic risk of reactive thrombocytosis in this clinical setting has not been delineated. Iron supplementation should be avoided to prevent undue elevation in hemoglobin and hematocrit levels and provide better phlebotomy control.

Thromboprophylaxis and symptomatic 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 preexisting clear indication or contraindication to aspirin therapy and demonstrated that low-dose aspirin (eg, 100 mg/d) reduces the rate of thrombosis and cardiovascular deaths in patients with PV receiving standard phlebotomy and supportive care. The aspirin-treated group suffered 60% fewer major thromboses and cardiovascular deaths (3.2% vs. 7.9% absolute incidence) after roughly 3 years of follow-up. Low-dose aspirin also can effectively control erythromelalgia and other vasomotor symptoms in most patients. PVSG trials showed that higher doses of aspirin (ie, 500-900 mg/d) offer no added benefit but increase the risk of bleeding complications, especially when combined with dipyridamole. Elderly PV patients on high-dose aspirin are at highest risk of bleeding. The ECLAP trial, however, observed only a modest increase in epistaxis and no increase in major bleeding on low-dose aspirin. The role of clopidogrel and other anticoagulants in thrombosis prevention in PV is not well defined and is not considered standard of care.

Because of the observations from the PVSG regarding early thrombotic risk, hydroxyurea should be used in high-risk patients at the start of aggressive phlebotomy (Table 16-10). In general, patients with PV should avoid practices that augment their hypercoagulability or risk of vascular complications, such as smoking and the use of oral contraceptives or estrogen hormone replacement therapy. For those requiring a surgical procedure, aggressive antithrombotic prophylaxis should be given postoperatively, in addition to ensuring that stable, normal hematocrit and platelet counts are maintained before surgery.

Puritus may be a particularly disturbing symptom that often is unresponsive to phlebotomy or antiplatelet therapy. Antihistamines, PUVA, cholestyramine, or selective serotonin

reuptake inhibitors (eg, paroxetine) may provide symptomatic relief. Cytoreductive therapy with hydroxyurea or interferon (IFN α or pegylated IFN) may help in refractory cases. Painful splenomegaly and unacceptable hypercatabolic symptoms usually require treatment with hydroxyurea or interferon (IFN α or pegylated IFN). Splenectomy or splenic irradiation may be necessary for palliation in selected patients who are intolerant of or unresponsive to cytoreductive agents.

Acute thrombosis management

Acute thrombotic events in patients with PV are managed with therapeutic systemic anticoagulation in a similar manner to other patients who present with acute thrombosis. It is also important to control the hematocrit and platelet count to minimize progression or recurrence; phlebotomy to normalize the hematocrit quickly should be initiated. The utility of plateletpheresis 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.

Cytoreductive therapy

The choice of cytoreductive agent is based on the patient's age, the need to treat painful splenomegaly or troublesome hypercatabolic and constitutional symptoms, and whether or not concomitant thrombocytosis is present. Hydroxyurea, a ribonucleotide reductase inhibitor, reduces the thrombosis rate, can normalize the platelet count and spleen size, and frequently ameliorates hypercatabolic symptoms. It is the first choice for most patients with PV requiring cytoreductive therapy. The mutagenic and leukemogenic potential of hydroxyurea has been the subject of significant concern, but overall, the AML/MDS risk with chronic hydroxyurea therapy appears to be low based on data from the ECLAP (PV) and United Kingdom Medical Research Council Primary Thrombocythemia 1 (PT-1; ET) trials. Nevertheless, because of uncertainty regarding these concerns, hydroxyurea 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 hydroxyurea include cytopenias and, less commonly, chronic mucocutaneous ulcers.

Subcutaneous injections of recombinant IFN α , at initial doses of 3-5 million IU three times per week (less in elderly patients), reliably control blood counts, splenomegaly, and constitutional symptoms in the majority of PV patients. IFN α therapy is safe during pregnancy, in contrast to

hydroxyurea, which may be teratogenic (although experience from sickle cell anemia populations suggests that hydroxyurea is a low-risk agent, so abortion is not justified solely based on inadvertent fetal hydroxyurea exposure). Adjusted-dose IFN α can be useful for late-stage patients with progressive splenomegaly and poorly controlled peripheral blood leukoerythroblastosis. In general, the inconvenience, cost, and chronic adverse effects (including anorexia, depression, and fatigue) limit the practical use of IFN α to women who desire pregnancy and to those infrequent patients who do not tolerate or do not respond to other agents. 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; current trials are aimed at assessing the efficacy and safety of pegylated IFN α in a larger cohort of PV patients.

Anagrelide, a prostaglandin synthetase inhibitor, selectively inhibits platelet production (although it can also cause mild anemia). It does not treat the hypercatabolic features of PV. The effect of anagrelide on thrombotic incidence has not yet been established clearly in patients with PV, and it should be considered a second-line agent. This is particularly true in light of data reported from the PT-1 trial comparing anagrelide and hydroxyurea in 809 patients with high-risk ET that suggested that anagrelide was associated with a higher risk of thrombosis compared with hydroxyurea (discussed later in this chapter), which has heightened concern. Studies of anagrelide in patients with ET have suggested that MPN-associated thrombotic complications are minimized if the platelet count is maintained $<400 \times 10^9/L$; without good prospective data, this goal should be considered for PV patients with thrombocytosis who are being treated with cytoreductive agents. Anagrelide does not affect fertility, but it crosses the placenta and is of uncertain teratogenic potential; therefore, it is contraindicated during pregnancy. Anagrelide is a vasodilator, so it must be used cautiously in elderly patients and those with heart disease. Common adverse effects, including headache, palpitations, diarrhea, and fluid retention, may be avoided or minimized by starting at a lower dose and titrating up.

Conventional alkylating agents generally are avoided in patients with PV. Chlorambucil clearly increases the risk of AML/MDS, and busulfan probably does so as well. Occasional patients with disease manifestations that are refractory to other cytoreductive treatments, especially elderly patients with a limited life expectancy, may benefit from a limited schedule of busulfan (eg, intermittent 2-week courses). Similar to chlorambucil, ^{32}P has been demonstrated in randomized trials to increase the risk of hematologic and nonhematologic malignancies, particularly when

combined with alkylating agents or hydroxyurea. It is well tolerated in the short term, however, and responses after a single treatment may last several months. Recent studies indicate that lower-dose ^{32}P may be equally effective as conventional doses, but with reduced and delayed risk of malignancy development. Therefore, low-dose ^{32}P is a reasonable palliative option for patients >70 years of age and may be especially useful in patients whose blood counts are difficult to control with hydroxyurea. Pipobroman is a neutral amide of piperazine, a metabolic competitor of pyrimidines that is related chemically to alkylating agents. Although unavailable in the United States since 1996, pipobroman appears to control PV disease manifestations as effectively as hydroxyurea. The ECLAP study, with shorter follow-up, revealed that pipobroman use was a significant risk factor for AML/MDS in PV, as had been shown previously for more conventional alkylating agents. None of the available cytoreductive therapies reliably modify established marrow fibrosis or prevent its progression in PV. Selective JAK2 TK inhibitors currently are undergoing clinical trials for PV and may supersede cytoreductive therapies or may be of value in patients refractory to or who are unable to tolerate hydroxyurea.

Hematopoietic SCT

Allogeneic SCT offers the potential to restore normal hematopoiesis with donor cells, reverse marrow fibrosis, and eradicate the malignant PV clone. Data for SCT in PV are derived from small studies of patients <65 years of age with rapidly progressive PV (ie, usually with clinicopathologic features indicating evolution to postpolycythemic myelofibrosis). Complete remissions and long-term survival are achievable after myeloablative allogeneic SCT from a histocompatible (human leukocyte antigen [HLA] matched) related or unrelated donor. Reported overall survival and nonrelapse mortality rates, however, vary widely among transplantation centers, likely reflecting differences in conditioning regimens, patient selection, and disease-related factors. Transplantation for untreated AML that has evolved from PV appears to be ineffective and cannot be recommended unless patients respond to induction or consolidation chemotherapy by returning to chronic-phase disease. There is little experience to date with outcomes of allogeneic transplantation after nonmyeloablative preparative regimens, although studies in PMF suggest that nonmyeloablative transplantation may offer significant efficacy with acceptable toxicity for patients with a related or unrelated donor. Currently, the high risks of morbidity and mortality with myeloablative allogeneic transplantation and the baseline favorable prognosis with PV restrict the use of SCT to younger patients with a poor prognosis.

Therapy for secondary AML in PV

Secondary AML arising from PV often, but not always, evolves during the postpolycythemic myelofibrosis stage of disease. AML in the setting of a previous diagnosis of PV usually is treated with conventional induction chemotherapy regimens. The response rate is low, and treatment-related morbidity and mortality are high, suggesting that these patients should be enrolled in clinical trials whenever possible. If remission is achieved, the duration usually is short lived. Allogeneic myeloablative SCT, however, can be considered during postinduction therapy remission in suitable younger patients with an acceptable donor. More generally, patients with MPNs who transform to AML do poorly for multiple reasons, including the preexisting marrow disorder and systemic complications associated with advanced disease, frequent leukemic cell chemotherapy resistance, and comorbidities of age and other medical conditions.

Pregnancy with PV

Because PV is uncommon in women of childbearing age, few pregnancy outcomes have been reported. One series of 18 pregnancies suggested poor outcomes unless the hematocrit was managed meticulously. Use of aspirin, phlebotomy, and, in some cases, low-molecular weight heparin throughout pregnancy and for several weeks postpartum resulted in good maternal–fetal outcomes. The optimal regimen is undefined. Because severe iron deficiency may cause low-birth weight and anemia in the neonate, some iron supplementation of phlebotomized women with PV may be indicated in selected cases during the first trimester of pregnancy. Because of the normal dilutional anemia of pregnancy, it is recommended that women with PV be phlebotomized to a hematocrit of <37% to minimize the risk of hyperviscosity, thrombotic, and hemorrhagic complications. Most of the myelosuppressive agents used for PV are contraindicated in pregnancy. If cytoreductive therapy is required, IFN α is the treatment of choice. It has been shown to be safe and effective in a small number of cases and is preferable to repeated apheresis procedures.

Key points

- PV is a stem cell disorder characterized by increased progenitor cell sensitivity to growth-promoting cytokines and is associated most commonly with activating somatic mutations in the JAK2 TK.
- PV (primary, absolute polycythemia) must be differentiated from relative and secondary polycythemias. Secondary polycythemias may be either physiologically appropriate (elevated EPO induced by tissue hypoxemia) or physiologically inappropriate (renal or ectopic overproduction of EPO or response to other

Key points (continued)

erythropoietic stimuli). Familial polycythemic states often are associated with truncating EPO receptor mutations (low endogenous EPO level) or VHL/HIF2A mutations (high endogenous EPO level).

- Unless erythrocytosis is an incidental finding, at diagnosis, patients with PV have symptoms related to hyperviscosity, hypercatabolism, microvascular events, or thromboembolic complications.
- The median survival time of PV exceeds 10 years when patients are managed appropriately; thrombosis and bleeding are the major causes of morbidity and mortality. Transformation to AML is uncommon but is associated with use of radiophosphorous or alkylating agents.
- Major risk factors for thrombosis with PV include uncontrolled erythrocytosis and thrombocytosis, age >60 years, and a history of prior thromboembolic events. Smoking, established cardiovascular disease, and inherited or acquired thrombotic diatheses are likely also important risk factors.
- The hematocrit and platelet count should be normalized for several weeks before elective surgery to minimize the risk of perioperative thrombosis and bleeding in patients with PV. Postoperative thrombosis prophylaxis should be given whenever possible.
- All patients with PV should be phlebotomized to maintain the hematocrit closer to a physiologic range (ie, <45% for men, <42% for women, and <37% during late pregnancy). Aspirin at a dose of ~100 mg/d should be routinely used for thrombosis prophylaxis, unless a clear contraindication exists. Myelosuppressive agents, splenectomy, and hematopoietic SCT are indicated for specific patient populations.

Essential thrombocythemia

Clinical case

A 40-year-old previously healthy female landscaper has been complaining to her husband about increased fatigue and numbness on her face and legs since about 3 weeks ago. She initially thought that maybe it was from the warm weather, but this seems to persist even while she is in an air-conditioned room and even after the weather has started to cool down. Despite the fatigue, she decided to go to a local blood banking center that day to donate blood, which she has been doing for 10 years now. Noticing that she is not feeling well, the local nurse advised her to get a routine CBC before she decides to donate blood. After 30 minutes, the nurse came back and told her that her platelet count is $1,000 \times 10^9/L$. She was asked by the nurse to see a hematologist. She was found to have the JAK2 V617F mutation. Following a bone marrow biopsy, the slides were reviewed and showed a hypercellular bone marrow (70% cellularity) with megakaryocytic hyperplasia with atypia. There was no mild reticulin fibrosis. FISH for BCR-ABL1 is negative. Iron studies and inflammatory markers (erythrocyte sedimentation rate levels and C-reactive protein levels) were within normal limits.

ET is the second-most-common MPN in the United States, with an annual incidence rate of approximately 0.5 cases per 100,000 persons per year. The median age at diagnosis is approximately 60 years, although the diagnosis increasingly is made in younger adults. Women with ET outnumber men 1.5- to 2-fold, particularly among ETs diagnosed in the third to fifth decade of life. Morbidity and mortality from ET predominantly relate to thromboembolic, vasomotor, and, less commonly, hemorrhagic complications.

Pathobiology

Clonality

A clonal hematopoietic population can be demonstrated in most, but not all, women with ET when evaluating for G6PD isoenzyme expression or for inactivation patterns of polymorphic alleles of X-linked genes in myeloid populations (ie, granulocytes, platelets, and/or hematopoietic progenitors) and can control somatic cell populations (usually T-lymphocytes, skin fibroblasts, or buccal mucosal cells). Constitutive skewing is noted in 20%-25% of normal women <50 years of age, and age-related skewing is present in >50% of women older than 75 years. Thus, these analyses of clonality are specific only for selected younger women. The inability to detect a clonal cell population in a portion of younger women with ET may relate to the infrequency of the affected clone as a proportion of total myeloid cells, restriction of clonal maturation to the megakaryocytic lineage, or alternative pathogenic mechanisms that lead to polyclonal platelet overproduction.

JAK2/MPL mutations

The JAK2 V617F mutation is present in at least 40%-50% of ET patients; using sensitive assays, it is possible to detect the mutant allele in different cell types (neutrophils, platelets) such that testing of peripheral blood neutrophils for the JAK2 V617F allele is appropriate as a diagnostic test. Patients with ET who have JAK2 V617F have a higher median hemoglobin concentration and neutrophil count than those who lack the mutation, and they may have more thromboembolic events and require more cytoreductive therapy. Marrow karyotype is normal in 90%-95% of cases. Recent studies have shown that 3%-5% of ET patients have somatic activating mutations in the *TPO receptor* (*MPL*). By comparison, germline *TPO* or *MPL* mutations that lead to constitutive overexpression of the gene product have been identified in several kindreds with hereditary thrombocytosis. Hereditary thrombocytosis is rare. There were also two reported cases of Medelian inheritance of non-V617F germline *JAK2* variants (*JAK2V617I* and *JAK2R564Q*) in two families with hereditary thrombocytosis.

Other biologic features

Biologic and functional abnormalities frequently are found in hematopoietic progenitor cells and platelets from ET patients. Myeloid progenitors demonstrate increased in vitro sensitivity to cytokines, with excessive colony growth in the majority of cases and endogenous megakaryocyte colony formation in a significant proportion of ET cases. A substantial proportion of ET patients demonstrate EEC growth, megakaryocytic progenitor sensitivity to TPO (the major megakaryocytic growth and differentiation factor), or both. Normal to elevated plasma levels of TPO are observed in most patients with ET, as well as in many patients with PV. Some earlier studies attributed the higher TPO levels to decreased expression of the c-Mpl receptor on megakaryocytes and platelets, with decreased platelet-mediated clearance of bound ligand. More recent studies have not confirmed those earlier findings, suggesting that low c-Mpl expression levels and high plasma TPO levels are not specific or consistent abnormalities in ET. Increased mean platelet volume and qualitative platelet aggregation defects are also found in up to 90% of patients with ET; however, they are not useful clinical predictors of the risk of thrombosis or bleeding. Recent studies have identified somatic mutations in *TET2* and *ASXL1* in a small subset of ET patients; however, these mutations are not specific for ET (vs. other myeloid neoplasms). Similar to PV, some emerging data suggest that the presence of *ASXL1* mutations in patients with ET may increase the propensity to transform to MF.

Clinical features

At least one-half of patients with ET are asymptomatic at diagnosis, but during the course of disease, vasomotor, thrombotic, or hemorrhagic manifestations eventually will occur in most individuals (Table 16-11). In contrast to PV, hypercatabolic signs and constitutional symptoms are uncommon in ET. Palpable splenomegaly is present in ~25% of ET patients at diagnosis, and ultrasound assessment reveals increased spleen length or volume in many patients with nonpalpable splenomegaly. Vasomotor manifestations, which appear to be due to platelet–endothelial interactions and inflammation in small arterioles, usually manifest as symptoms in the central nervous system or acral extremities (Table 16-11). In ET, thrombosis in the arterial system, including the cerebral, coronary, and peripheral arteries, is roughly three times more common than thromboembolic complications involving the veins. Approximately 10%-25% of patients have a history of a thromboembolic event at the time they are diagnosed with ET. Hemorrhage is reported in only 6% of patients at diagnosis, most commonly as gastrointestinal or oral mucosal bleeding. Bleeding is more

Table 16-11 Clinical features of essential thrombocythemia.

Vasomotor	“Vascular” headaches, visual disturbances, dizziness, burning dysesthesia of the palms and soles (erythromelalgia), acrocyanosis, paresthesias, cutaneous ulcers, cognitive or psychiatric deficits, seizures
Thrombotic	Arterial: cerebral (TIA, CVA), coronary, ophthalmic, distal/ extremities Venous: deep extremities, pelvic, mesenteric, hepatic, portal
Hemorrhagic	Gastrointestinal, mucosal, epistaxis, urogenital, deep hematoma, hemarthrosis
Obstetric	First-trimester spontaneous abortion

CVA = cerebrovascular accident; TIA = transient ischemic attack.

Multiple sources.

common in older individuals and in patients with platelet counts $>1,500 \times 10^9/L$. The bleeding risk with a platelet count $>1,500 \times 10^9/L$ may be due, at least in some cases, to acquired vWD or to high-dose aspirin use.

ET is discovered as an incidental laboratory abnormality in 70% of patients who are diagnosed <40 years of age. Upon careful questioning, however, less than one-fourth of these individuals are truly completely asymptomatic, although the connection of nonspecific symptoms, such as headaches or episodic tinnitus, to ET is not always clear. Approximately 4%-10% of younger patients with ET have suffered a thrombotic or bleeding complication prior to diagnosis. As illustrated by the previous clinical case, young women with ET have a significantly increased risk of first-trimester abortions (up to 36% of documented pregnancies) and may suffer recurrent fetal loss; studies have not identified clinical or laboratory risk factors for pregnancy-associated complications in ET with the exception of poorly managed platelet counts.

Differential diagnosis and laboratory features

Distinction from reactive thrombocytosis or other myeloid disorders

The diagnosis of ET as the cause of persistent thrombocytosis (ie, $>450 \times 10^9/L$) relies on the exclusion of disorders associated with reactive thrombocytosis and the exclusion of other MPNs (especially PV, PMF, and CML) and MDS [especially 5q- syndrome, chromosome 3(q21;q26) abnormalities, or refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T)]. The revised WHO criteria lowered the threshold platelet count for ET to $450 \times 10^9/L$ (Table 16-12). A diagnosis of ET according to the

Table 16-12 Diagnostic criteria for essential thrombocythemia.

Disease	Essential thrombocythemia
Major	<ol style="list-style-type: none"> 1. Sustained platelet count $\geq 450 \times 10^9/L$ 2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes; no significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis 3. Not meeting World Health Organization criteria for polycythemia vera, primary myelofibrosis, chronic myelogenous leukemia, myelodysplastic syndrome, or other myeloid neoplasm 4. Demonstration of JAK2 V617F or other clonal marker, or in the absence of a clonal marker, no evidence for reactive thrombocytosis
Diagnosis	Requires meeting all four major criteria
	Adapted from Swerdlow SH, Campo E, Harris NL, et al., eds. <i>World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues</i> . Lyon, France: IARC Press; 2008.

revised WHO requires four features: thrombocytosis; bone marrow findings, including megakaryocyte dysmorphology; absence of evidence of another clonal myeloid disorder; and the presence of a clonal marker (JAK2 or MPL mutation or karyotypic abnormalities) or absence of reactive thrombocytosis. Iron deficiency, infection, inflammation, surgery, trauma, tissue injury or infarction, malignancy, and post-splenectomy state all can cause secondary or reactive thrombocytosis because platelets are an acute-phase reactant. Increased levels of inflammatory mediators, including IL-1b and IL-6 in addition to IL-11 (a direct thrombopoietic stimulator), have been associated with reactive thrombocytosis. An elevated C-reactive protein measurement is a surrogate marker for increased levels of IL-6, which can suggest an occult inflammatory process.

Laboratory features

The LAP score typically is elevated in ET, like PV but unlike CML. Moderate leukocytosis may be found in up to one-half of ET patients. The peripheral blood smear often is notable for large or giant platelets (Figure 16-5) with occasional eosinophils, basophils, or circulating megakaryocyte fragments. Howell-Jolly bodies suggest hypoplasia; if the cause of this is not obvious (eg, prior splenectomy), then celiac disease, amyloidosis, or a hemoglobinopathy resulting in functional hypoplasia should be considered.

Because JAK2 and MPL mutations are specific for clonal hematologic disease, their presence is valuable in excluding purely reactive thrombocytosis. Given that as many as 30%-40% of ET patients are negative for JAK2/MPL mutations, however, a negative JAK2 result does not exclude a diagnosis

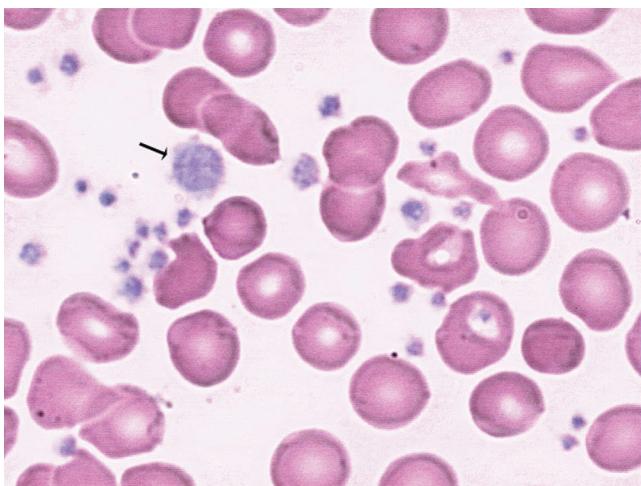


Figure 16-5 Increased numbers of platelets, including abnormally large platelets (arrow), are characteristic of essential thrombocythemia. Similar platelet abnormalities may also be seen in other myeloproliferative disorders. From American Society of Hematology Image Bank, #100445.

of ET. The absolute value of the platelet count usually does not help distinguish reactive thrombocytosis from ET, although reactive conditions infrequently cause elevations in platelet counts of $>2,000 \times 10^9/L$.

The significance of blood leukoerythroblastic features and their possible association with PMF rather than ET has been investigated. One study suggested that patients with more prominent circulating immature cells, teardrop erythrocytes, mild splenomegaly, and stainable marrow reticulin fibrosis more commonly progressed to overt myelofibrosis and had a significantly shorter survival, suggesting that their natural histories were more consistent with PMF and that they might have been classified as having “prefibrotic” or “early fibrotic” PMF rather than ET. This area is complex and requires an experienced morphologist and clinical correlation. Moreover, although patients with evidence of leukoerythroblastosis may be at higher risk for progression to post-ET myelofibrosis, the diagnostic and therapeutic strategy for patients with clonal thrombocytosis does not differ based on the likelihood for progression to overt myelofibrosis.

Bone marrow and cytogenetic findings

Marrow evaluation is important in suspected ET cases to assess for histopathologic features that are characteristic of ET to confirm the presence of adequate iron stores and to assess for fibrosis or other atypical features that might suggest an alternative diagnosis. Increased numbers and clusters of large megakaryocytes with hyperploid nuclei are seen in ~90% of marrow samples from patients with ET, and the bone marrow may be normocellular or only mildly hypercellular.

Trilineage dysplasia and significant reticulin and collagen fibrosis are commonly minimal or absent. If >15% ringed sideroblasts are present, the rare provisional entity of RARS-T rather than ET must be considered, although the prognostic importance of this distinction is unclear. The presence of trilineage dysplastic morphologic features should raise the suspicion of MDS.

Alternative disorders to ET are particularly important to recognize in younger patients who might benefit from targeted therapy with imatinib (CML), treatment with lenalidomide [del(5q) MDS], or an early SCT if the diagnosis is revised. Cytogenetic studies are helpful in excluding CML or MDS (especially the 5q- syndrome), and specific assessment of *BCR-ABL* should be undertaken because CML can present with isolated thrombocytosis and a normal WBC count. Patients with isolated thrombocytosis in association with the t(9;22)(q34;q11) translocation detected on routine karyotyping or large proportions of cells positive for *BCR-ABL* detected by FISH follow a disease course similar to that of CML. Thus, such patients should be considered to have atypical CML and should be managed accordingly. The meaning of low-level *BCR-ABL* signals detected only by sensitive PCR assays in patients with suspected ET is less clear, however. *JAK2/MPL* mutational status or karyotypic abnormalities described in ET do not appear to have prognostic significance.

Course and prognosis

Disease progression

A single-institution observational study of 322 ET patients with a median follow-up time of 13.6 years showed that life expectancy is similar to age-matched controls in the first decade after diagnosis, but overall survival became significantly worse thereafter. Age at diagnosis of 60 years or older, leukocytosis, ongoing tobacco use, and diabetes mellitus were independent predictors of poor survival. Nevertheless, many patients can expect a normal or near-normal life span if major thrombotic or bleeding complications are avoided. Several studies have reported that transformation to AML is rare in the natural course of ET (<2%) in the first decade after diagnosis; however, the risk of leukemic progression or any myeloid disease transformation increases substantially in the second (8.1% and 28.3%, respectively) and third (24.0% and 58.5%, respectively) decades after diagnosis. It does not appear that hydroxyurea treatment by itself increases the risk of leukemic transformation; however, the AML transformation rate has been reported at 3.5%-10% at 4-10 years when hydroxyurea is used in the setting of underlying cytogenetic abnormalities, marrow fibrosis, or concomitant use of additional chemotherapy agents.

Approximately 5% of patients with apparent ET with isolated thrombocytosis at diagnosis will later develop erythrocytosis consistent with transformation to PV; this most commonly occurs in patients with *JAK2* V617F because acquisition of *JAK2*V617F mutations after ET diagnosis does not commonly occur. Alternatively, 2%-6% of patients progress to develop post-ET myelofibrosis, a disease that is clinically and pathologically indistinguishable from PMF. As noted, such patients may have had a prefibrotic or early fibrotic stage of PMF, rather than true ET. In general, these observations illustrate the overlapping clinical and pathophysiological features of MPNs.

Thrombohemorrhagic risk

Between 10% and 50% of patients with ET will have a thrombotic episode during the first decade after diagnosis, and 4% will suffer a hemorrhagic complication. Older age and previous history of thrombohemorrhagic complications are the major risk factors in most studies. Complications of either type are uncommon in patients <40 years of age. One prospective observational study found no significant increase in the risk of thrombosis (1.91 cases per 100 patient-years, with 4.1 years of median follow-up) among untreated ET patients <60 years of age with a negative prior thrombohemorrhagic event history and a platelet count <1,500 × 10⁹/L when compared with age-matched healthy controls. Older age and a platelet count >1,500 × 10⁹/L have been cited as predictors of bleeding risk in ET; however, the influence of comorbid factors must be considered (Table 16-13). Some ET patients with uncontrolled thrombocytosis and clinical bleeding are found to have vWD, as described previously for PV.

The overall risk of thrombotic complications in ET can be stratified based on age, history of prior thrombosis, and the presence of additional cardiovascular risk factors (Table 16-13). The relative importance of associated cardiovascular risk factors with arterial thromboembolism has not been

clearly demonstrated; however, ET patients with a history of cigarette smoking, diabetes mellitus, or hypercholesterolemia are at higher risk of cerebral or cardiac thrombotic events. Although limited data exist, most experts consider oral contraceptive use, estrogen replacement therapy, and other acquired and congenital prothrombotic conditions to be modifiers of thrombotic risk with ET. Specifically, the presence of genetic thrombophilic states, including activated protein C resistance, prothrombin 20210A, or less common thrombophilic states (protein C/S deficiency, ATIII deficiency, or antiphospholipid antibodies), imparts a higher risk of thrombosis with ET. Although some studies have found an increased risk of bleeding in ET patients with a platelet count of 1,500 × 10⁹/L, no data suggest that absolute platelet number is clearly predictive of thromboembolic complications in the absence of other clinical risk factors. Nevertheless, controlling the platelet count may be protective, and studies with hydroxyurea (targeted at maintaining the platelet count at <600 × 10⁹/L) and anagrelide (targeted at maintaining the platelet count at <400 × 10⁹/L) have shown that these drugs decrease the risk of primary or recurrent thrombotic events (from 24%-36% during the 27-month observation period).

Therapy

General considerations

Treatment approaches for ET are based on the individualized risks for thrombosis or bleeding, the presence of vasomotor symptoms, and the risks and benefits of the available platelet-lowering agents (Table 16-14). The three platelet-lowering agents commonly used for ET in the United States are hydroxyurea, anagrelide, and IFNα. Alkylating agents are almost never used in ET because of their proven leukemogenicity in PV.

Cytoreductive therapy

Two important randomized trials of high-risk patients with ET inform current management principles. The first

Table 16-13 Risk stratification for thrombotic and hemorrhagic complications of essential thrombocythemia.

Low risk (all of the following)

- Age <60 years old
- No history of thromboembolism
- No cardiovascular risk factors (smoking, hypercholesterolemia)

Indeterminate risk

- Neither low- nor high-risk disease

High risk (one or both)

- Age ≥60 years old
- History of thromboembolism

Adapted and modified from Tefferi A. Recent progress in the pathogenesis and management of essential thrombocythemia. *Leuk Res.* 2001;25:369-377.

Table 16-14 Current treatment of essential thrombocythemia.

Risk categories	Essential thrombocythemia
Low	Low-dose aspirin
Intermediate	Low-dose aspirin*
High	Low-dose aspirin + hydroxyurea

* Clinically significant acquired von Willebrand disease should be excluded before the use of aspirin in patients with platelet count >1,000 × 10⁹/L.

From Vanucchi AM, Guglielmelli P, Tefferi A. Advances in understanding and management of myeloproliferative neoplasms. *CA Cancer J Clin.* 2009;59:171-191.

study from Italy, a randomized trial of 114 patients, convincingly showed a role for hydroxyurea in decreasing thromboembolic events in high-risk ET patients who either are >60 years of age, have a previous history of thromboembolism, or both. A recent follow-up report of this study (median treatment time, 73 months) revealed a continued benefit for hydroxyurea: 45% of patients in the control group suffered a thrombotic event versus 9% of patients in the hydroxyurea group. Of note, 1.7% of control patients and 3.9% of the group receiving hydroxyurea developed secondary myeloid malignancies (AML/MDS), a difference that was not statistically significant. The second important randomized study in ET was the PT-1 trial. A total of 809 patients with ET at high risk of thrombosis were enrolled in the PT-1 study, received low-dose aspirin (75–100 mg daily), and were randomized to receive either hydroxyurea or anagrelide, with a goal platelet count of $<400 \times 10^9/L$. After a median follow-up of 39 months, patients in the anagrelide group were significantly more likely than those in the hydroxyurea group to have reached the adverse primary endpoint of thrombosis or serious hemorrhage. Specifically, compared with hydroxyurea plus aspirin, patients receiving anagrelide plus aspirin had increased rates of arterial thrombosis, serious hemorrhage, and development of marrow fibrosis but a decreased rate of venous thromboembolism. Patients receiving anagrelide were more likely to withdraw from their assigned treatment because of toxicity or treatment failure. Taken together, these two studies suggest that patients with ET at high risk of thrombosis should be treated with low-dose aspirin and hydroxyurea with a goal platelet count of $<400 \times 10^9/L$ to $600 \times 10^9/L$. Anagrelide should be reserved for patients with an insufficient response to hydroxyurea or with intolerable hydroxyurea-associated adverse effects. The use of hydroxyurea or anagrelide in patients at lower risk of thrombosis or the use of other cytoreductive agents in ET patients in general is not clear. Recent studies have reported excellent safety and efficacy of pegylated IFN α in ET, although this has not been assessed in randomized clinical trials.

Pregnancy

On the basis of case reports demonstrating safety and efficacy, IFN α remains the cytoreductive agent of choice for symptomatic or otherwise high-risk young women with ET (eg, those with thrombotic history) who desire pregnancy. Pegylated IFN α appears to have a more favorable safety and efficacy profile and should be considered as an alternative therapeutic regimen in pregnant women with ET. Although pregnancy is often successful in asymptomatic women with untreated ET, the increased risk of first-trimester abortions

does not appear to be affected by the use of platelet-lowering agents or by prophylactic platelet depletion with apheresis. In addition, the outcome of a subsequent pregnancy is not predicted by the outcome of a first pregnancy. Aspirin commonly is recommended for women with ET and prior fetal loss; however, the benefit of this intervention has not been proven formally.

Prevention and management of thrombosis and hemorrhage

Low-dose aspirin as primary prophylaxis for ET patients is recommended in view of the beneficial results with aspirin in PV in the ECLAP study and the favorable outcomes of patients treated with aspirin and hydroxyurea in the PT-1 trial. In the setting of acute arterial or venous events, emergency plateletpheresis occasionally may be indicated to reduce the platelet count if it is very high. Standard heparin and warfarin therapy are indicated for venous thrombosis as for non-ET patients who present with thrombosis. Large-vessel arterial thrombotic events may require acute intervention with a heparin or thrombolysis. Lifelong warfarin is reserved for patients with recurrent events or if the primary event was catastrophic and unprovoked in a patient with a normal platelet count on cytoreductive therapy. In either circumstance, the patient should be monitored closely for bleeding while receiving anticoagulation. Platelet-lowering agents should be used to maintain the platelet count in a safe range, ideally $<400 \times 10^9/L$. Aspirin is indicated following an arterial thromboembolic event if the patient is not already taking it. As with PV, the risk of bleeding in ET appears to be elevated with high doses (>325 mg/d) of aspirin. Therefore, lower aspirin doses, in the range of 75–300 mg/d, normally are advised. As for PV, the role of clopidogrel or other antiplatelet agents in ET is unknown. The usefulness of aspirin after a venous thrombotic event is more questionable, and aspirin treatment should be deferred until warfarin therapy is discontinued. As in the case of PV, the platelet count in patients with ET should be normalized for several weeks before elective surgery to minimize the risk of perioperative thrombosis and bleeding.

SCT or transformation to acute leukemia

Despite the generally favorable prognosis with ET, occasional patients evolve to extensive myelofibrosis with myeloid metaplasia or AML. Allogeneic SCT is feasible and beneficial for selected high-risk younger patients who have developed myelofibrosis and have a suitable related or unrelated stem cell donor. Only small series have been reported. As with PV, the roles for nonmyeloablative SCT or autologous SCT

remain undefined for high-risk ET. As in PV, patients with ET who transform to AML should be treated with standard induction and consolidation chemotherapy when clinically indicated. Patients with ET that transformed to AML who achieved first complete remission or who achieved a second chronic phase subsequently should undergo an allogeneic SCT if feasible, given the poor prognosis.

Key points

- ET is a diagnosis of exclusion after evaluating patients for other myeloid disorders and for reactive thrombocytosis. Reactive or secondary thrombocytosis may be due to iron deficiency, infection, inflammation, tissue injury or trauma, malignancy, and postsplenectomy state or functional hyposplenism. The platelet count alone does not distinguish reactive thrombocytosis from ET.
- *JAK2* and *MPL* mutations are present in 50%-60% of ET patients; their presence proves the existence of a clonal myeloid disorder but is not specific for ET, and their absence does not exclude a diagnosis of ET.
- The life expectancy of patients with ET is longer than for those with other MPNs, but patients are at risk for ET-related morbidity and mortality over time. Symptomatic bleeding, thromboembolic events (arterial > venous), and vasomotor complications affect most individuals eventually and are the major causes of morbidity and mortality.
- Risk factors for bleeding and thrombosis should be identified and considered in a risk-based treatment approach for patients with ET.
- Platelet-lowering agents and low-dose aspirin are indicated for high-risk patients with ET. Hydroxyurea should be the first choice because it is superior to anagrelide at preventing most thrombohemorrhagic events. Anagrelide can be a useful second-line agent. Low-dose aspirin alone should be used for low-risk patients and may effectively treat vasomotor symptoms.
- ET is the most common MPN detected in young women and is associated with recurrent fetal loss. Aspirin and, if cytoreduction is required, IFN α can be used to strive for a favorable pregnancy outcome.

Primary myelofibrosis

Clinical case

A 71-year-old male with a prior history of prostate cancer diagnosed 5 years ago status post (s/p) radical prostatectomy, gout, and cholecystitis is complaining of early satiety, discomfort over the left upper quadrant of the abdomen, night sweats, intermittent fevers, and chills. He was concerned that the prostate cancer may have come back so he immediately saw his oncologist. Physical examination revealed an enlarged spleen (20 cm below the left subcostal margin) and an enlarged liver. A CBC was requested and showed leukocytosis (white blood cell [WBC] = $21 \times 10^9/L$), normocytic anemia (Hgb = 10.7, MCV = 88 fl), plt = $292 \times 10^9/L$.

Clinical case (continued)

The WBC differentials includes neutrophils (%) = 67, lymphocytes (%) = 11, monocytes (%) = 1, basophils (%) = 2. Review of the peripheral blood smear shows some circulating blasts, teardrop cells, nucleated RBCs, and immature WBC. The *JAK2* V617F analysis was negative. Metaphase cytogenetics showed 46, XY [20]. *MPL* mutation analysis was positive. A bone marrow biopsy showed a hypercellular marrow with megakaryocytic hyperplasia and atypia with grade 4+4 reticulin fibrosis. He was started on ruxolitinib.

PMF is the least common of the four major MPNs and carries the worst prognosis. The annual incidence is reported at 0.2 cases per 100,000 persons per year, with a predominance of men >50 years of age. The median survival time is 3.5-5.5 years, although the natural history of PMF is quite variable depending on the presence or absence of poor prognostic features. A subset of low-risk patients will live longer than 10 years and require minimal active management (see further details later in the chapter). 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.

Pathobiology

Marrow microenvironment, CD34¹ cells, and clonality

The hallmarks of PMF are marrow fibrosis and extramedullary hematopoiesis; the latter most commonly affects the liver and spleen. These processes result from the proliferation and emigration of neoplastic hematopoietic cells and production of cytokines within the marrow microenvironment, leading to reactive proliferation of fibroblasts and other mesenchymal cells. Throughout all stages of disease, the circulating populations of red blood cells, granulocytes, and platelets are clonal. The number of circulating hematopoietic progenitors (CD34¹ cells) is increased significantly in PMF. This also is true for other MPNs, but the number of circulating CD34¹ cells in PMF patients can be 50-fold higher than in PV or ET. Higher levels of circulating CD34¹ cells in PMF are associated with more advanced bone marrow fibrosis and other disease characteristics. One study observed shorter survival and earlier transformation to AML among PMF patients with circulating CD34¹ cell counts <300/mL. A hostile marrow microenvironment alone does not appear to account for elevated CD34¹ counts because extensive myelofibrosis secondary to marrow involvement with carcinoma or lymphoid malignancies is associated only mild increases in the numbers of circulating progenitor cells.

The marked reactive mesenchymal cell proliferation in PMF has been linked to inflammatory response cytokines and megakaryocyte- and monocyte-derived growth factors.

Platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) are constitutively overproduced by clonal megakaryocytes in PMF. Platelets and monocytes or macrophages in PMF marrow release increased amounts of transforming growth factor b (TGFb), which also contributes to the stromal reaction. In PMF patients, elevated levels of IL-1 and tumor necrosis factor (TNF) are associated with augmented production or release of PDGF, bFGF, angiogenic factors such as vascular endothelial growth factor (VEGF), and osteogenic cytokines, in addition to direct or indirect effects of TGFb. Neoangiogenesis is more significant in PMF compared with other MPNs, and high marrow microvascular density is associated with advanced splenomegaly and shorter survival.

Cytogenetic and molecular findings

JAK2 V617F is found in ~50% of patients with PMF. Several series have reported an association between JAK2 V617F and elevated WBC count, older age, history of thrombosis, or pruritus. As in ET, approximately 5%-10% of patients with PMF have somatic *MPL* mutations, which activate *JAK2* in a subset of *JAK2* V617F-negative PMF patients. However, 30%-50% of PMF patients are negative for *JAK2/MPL* mutations, suggesting unidentified mutations activate signaling and are responsible for clonal hematopoiesis in this subset of PMF. Mutations of the *p53* gene, the *RAS* family of proto-oncogenes, *p16*, or *KIT* have been described in a minority of PMF cases; whether these mutations represent disease-initiating events in PMF or are acquired during PMF disease progression are not known. Recently, mutations involving genes important in RNA splicing particularly *SF3B1*, *U2AF1*, *ZRSR2*, and *SRSF2* also were identified in MF. Cytogenetic abnormalities are found in approximately one-third to one-half of patients with PMF at diagnosis and in approximately 90% at the time of disease progression to AML. The majority of patients with abnormal karyotypes have del(13q), del(20q), trisomy 8, trisomy 9, del(12p), or trisomy 1q. Pathobiologically relevant genes underlying these rearrangements generally are unknown.

Clinical features

Two-thirds of patients with PMF are symptomatic at diagnosis, predominantly with constitutional complaints related to a cytokine-mediated hypercatabolic physiologic state (Table 16-15). As illustrated in the previous clinical case, fevers, night sweats, and weight loss are frequent hypercatabolic signs. Severe fatigue is the most common symptom in patients with PMF. Hyperuricemia is also common and results from increased myeloid cell turnover; gout or renal complications of hyperuricemia can develop. Splenomegaly

Table 16-15 Clinical features of primary myelofibrosis.

Mechanism	Symptoms
Hypercatabolic state (cytokine related)	Fatigue, weight loss, nocturnal sweating, pruritus
Splenomegaly	Pain, early satiety, diarrhea
Anemia	Dyspnea, palpitations, light-headedness
Portal hypertension/ascites	Abdominal pressure, peripheral edema
Splenic infarct	Acute left upper quadrant pain, fever, nausea, subscapular pain
Esophageal varices/hemorrhoids	GI bleeding (melena or hematochezia)
Hypertrophic osteoarthropathy, periostitis	Bone and musculoskeletal pain
Ectopic myeloid metaplasia	Tumor mass effect (lung, GI, GU, CNS, spine)
Thrombocytopenia/platelet dysfunction	Bleeding, bruising
Hyperuricemia	Monoarticular arthritis, nephrolithiasis (synovitis, hematuria)

CNS = central nervous system; GI = gastrointestinal;

GU = genitourinary.

Multiple sources.

is found in 85%-100% of PMF patients at diagnosis. Ultimately, 35% of PMF patients will develop massive (ie, extending to the pelvic brim) splenomegaly. Extramedullary hematopoiesis in the spleen and, to a lesser degree, upstream effects of portal hypertension in the liver are responsible for the splenic enlargement. Extramedullary hematopoiesis also can cause hepatomegaly and lymphadenopathy. Rare patients with PMF develop nonhepatosplenic extramedullary hematopoiesis in locations such as the vertebral column (paraspinal or intraspinal lesions, which can lead to cord compression), lung, pleura, retroperitoneum, eye, kidney, bladder, mesentery, and skin. Lung extramedullary hematopoiesis is associated with pulmonary hypertension and marked reduction in overall survival and impaired quality of life.

Portal hypertension with ascites and varices develops in up to 7% of patients with PMF. This complication arises from thrombotic vasculopathy involving the portal circulation and extramedullary hematopoiesis. Portal hypertension and massive splenomegaly predispose to splenic infarction. Splenic infarction should be suspected in a patient with PMF who presents with acute or subacute left upper-quadrant pain radiating to the shoulder, with or without associated nausea and fever. Splenic infarcts do not have clear prognostic value. Pulmonary hypertension can occur as a complication of PMF and sometimes is under-recognized.

Anemia is the major hematologic complication of PMF. The cause of anemia in PMF is often multifactorial, including impaired erythropoiesis, hematopoietic failure, hemolysis, hemorrhage (usually gastrointestinal), and hypersplenism. Symptoms due to anemia are common among patients with PMF (Table 16-15). Approximately 50%-70% of patients are anemic at presentation, and 25% of patients have hemoglobin levels <8 g/dL. Progressive thrombocytopenia, usually in the setting of hematopoietic failure and hypersplenism (ie, sequestration and destruction of circulating platelets in the spleen), significantly increases the risk of bleeding. Low-grade disseminated intravascular coagulopathy may arise in some patients and can exacerbate the thrombotic and hemorrhagic risk. Secondary iron overload may develop because of inappropriate iron loading by the gut and red blood cell transfusion dependency. Autoimmune complications have been described with PMF, including hemolytic anemia and vasculitis.

Diagnosis

Diagnostic features have been published by the WHO (Table 16-16) that incorporate molecular, histopathologic, clinical, and laboratory features that distinguish PMF from other myeloid and nonmyeloid diseases. In most cases, the diagnosis of PMF is satisfied by finding increased bone marrow reticulin or collagen fibrosis, leukoerythroblastotic peripheral blood findings, and splenomegaly, in the absence of a secondary cause for these findings or of features better fitting ET or PV. CML should always be ruled out by molecular studies for the *BCR-ABL* rearrangement. *JAK2* and *MPL* mutations demonstrate the presence of clonal hematopoiesis, but their presence is not specific for PMF, and their absence does not exclude a diagnosis of PMF. Acute

myelofibrosis resulting from AML with megakaryocytic differentiation (ie, M7 AML by French-American-British [FAB] criteria and acute megakaryoblastic leukemia by WHO classification criteria) or other primary myeloid disorders may be confused with PMF. In most cases, acute megakaryoblastic leukemia is distinguished by a rapid disease onset, pancytopenia, mild splenomegaly, and a high frequency of marrow myeloblasts with a megakaryocytic immunophenotype (eg, positive for CD61). Many children who are diagnosed with PMF probably have acute megakaryoblastic leukemia. Other cases of acute myelofibrosis have a similar marrow histopathology and fulminant disease course to M7 AML but lack the 20% blast count required for the diagnosis of AML.

Patients with MDS may present with fibrosis in their bone marrow but usually lack the prominent splenomegaly and peripheral blood leukoerythroblastosis typical of PMF. The marrow in MDS with fibrosis should reveal trilineage dysplasia without osteosclerosis. Clonal abnormalities of chromosome 5 or 7 may be detected in either MDS or PMF and thus do not exclude a diagnosis of PMF. Late-stage PV or ET, where marrow fibrosis and myeloid metaplasia have developed, may be indistinguishable from PMF by morphologic and clinical criteria. The biology, prognosis, and treatment of PMF and postpolycythemic myelofibrosis or ET are identical. Other malignant and nonmalignant causes of marrow fibrosis are listed in Table 16-17. Of note, secondary marrow changes of increased reticulin fibrosis with megakaryocytic hyperplasia resembling PMF have been associated with systemic lupus erythematosus, Sjögren's syndrome, psoriatic arthritis, and other chronic autoimmune diseases, most commonly in the absence of splenomegaly or significant leukoerythroblastotic peripheral blood findings. These patients frequently have constitutional symptoms and anemia with

Table 16-16 Diagnostic criteria for primary myelofibrosis.

Disease	Criteria
Major	<ol style="list-style-type: none">1. Presence of megakaryocyte proliferation and atypia, usually accompanied by either reticulin and/or collagen fibrosis, <i>or</i>, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (ie, prefibrotic cellular-phase disease).2. Not meeting World Health Organization criteria for polycythemia vera, chronic myelogenous leukemia, myelodysplastic syndrome, or other myeloid neoplasm3. Demonstration of <i>JAK2</i> V617F or other clonal marker (eg, <i>MPL</i> W515L/K), <i>or</i>, in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases
Minor	<ol style="list-style-type: none">1. Leukoerythroblastosis2. Increase in serum lactate dehydrogenase level3. Anemia4. Palpable splenomegaly
Diagnosis	Diagnosis requires meeting all three major criteria and two minor criteria.

Adapted from Swerdlow SH, Campo E, Harris NL, et al., eds. *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008.

positive antinuclear antibody and direct antiglobulin titers. Clinical, hematologic, and marrow fibrotic abnormalities in these conditions often respond to corticosteroids.

Laboratory features

In addition to a high frequency of anemia, patients with PMF present with leukocytosis, leukopenia, thrombocytosis, or thrombocytopenia in 50%, 7%, 28%, and 37% of cases, respectively. Egress of immature cells from the marrow into the blood is characteristic of this disorder. Erythroid precursors may account for up to 20% of the circulating nucleated cells, and circulating blast cells are found in up to 30% of cases at diagnosis (blasts of $\geq 20\%$ indicates AML). In most cases, the peripheral blood smear reveals the typical leukoerythroblastic features of immature myeloid cells, nucleated red blood cells, teardrop erythrocytes, and large platelets. Although these findings are sensitive for the diagnosis of PMF, they are not highly specific. Secondary causes of marrow fibrosis (Table 16-17) also may give rise to a similar picture of peripheral blood leukoerythroblastosis. The LAP score in patients with PMF usually is elevated, although it may be normal or low in up to one-fourth of patients. Because of the high marrow cell turnover, LDH, bilirubin, and uric acid levels commonly are increased as well. Haptoglobin levels may be decreased, and there may be other clinical and laboratory indicators of low-grade idiopathic hemolysis.

Table 16-17 Differential diagnosis of primary myelofibrosis.

Acute myelofibrosis (acute megakaryoblastic leukemia, AML-M7)
Myelodysplasia with fibrosis
Late-stage PV, ET, or CML with evolution to myelofibrosis
Malignant causes of secondary 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
Nonmalignant causes of secondary myelofibrosis
Granulomatous infections (tuberculosis, histoplasmosis)
Paget disease
Autoimmune disorders (eg, systemic lupus, Sjögren's syndrome, psoriatic arthritis, primary autoimmune myelofibrosis)

CML = chronic myelogenous leukemia; ET = essential thrombocythemia; PV = polycythemic vera.

Clinical, morphologic, and histopathologic features

Approximately 20%-30% of patients with PMF are believed to present in the prefibrotic stage. The early prefibrotic stage of PMF, also referred to as the “cellular” or “proliferative” stage, may be associated with thrombocytosis and modest leukoerythroblastosis but may be difficult to distinguish from ET. This condition may cause diagnostic difficulties in the absence of clear evidence of megakaryocyte atypia on bone marrow biopsy. Splenomegaly may be present, and the marrow reveals hypercellularity, left-shifted myeloid maturation, and increased megakaryocyte numbers with clustering and nuclear dysplasia. The fibrotic stage of PMF is associated with reticulin or collagen fibrosis in addition to more characteristic clinical and peripheral blood changes and more prominent megakaryocyte atypia (Figure 16-6). With increasing degrees of fibrosis, a diagnostic marrow aspirate often is unobtainable, yielding a “dry tap.” Progressive medullary 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 is completely replaced by fibroblasts and extracellular matrix material (Figure 16-6). Osteosclerosis may develop in some cases.

Course and prognosis

The outlook for PMF is more variable than for other MPNs. A number of studies have identified clinical and laboratory features that predict a more aggressive disease course and shorter survival in patients with PMF (Table 16-18). In general, morbidity and mortality are related to hematopoietic failure, thrombosis, hypersplenism, advanced age, and evolution to AML. AML is the cause of death in 5%-30% of patients. The risk of developing AML is increased among patients with severe anemia and a high number of circulating immature myeloid cells.

A hemoglobin < 10 g/dL is the most consistent adverse prognostic indicator for patients with PMF. Age also appears to be important; patients < 55 years of age at diagnosis have a median survival of 8-10 years, whereas the median survival of older patients (variously defined as > 55 , > 60 , or > 65 years of age) is 3-5 years in most studies. The presence of constitutional symptoms, increased circulating peripheral blasts, and elevated WBC ($> 25 \times 10^9/L$) also contribute to poor outcomes in MF. These clinicopathologic factors became the basis for the development of the International Prognostic Scoring System (IPSS) for myelofibrosis. The IPSS, however, was designed primarily to evaluate prognosis only at the time of original diagnosis. A subsequent prognostic scoring system

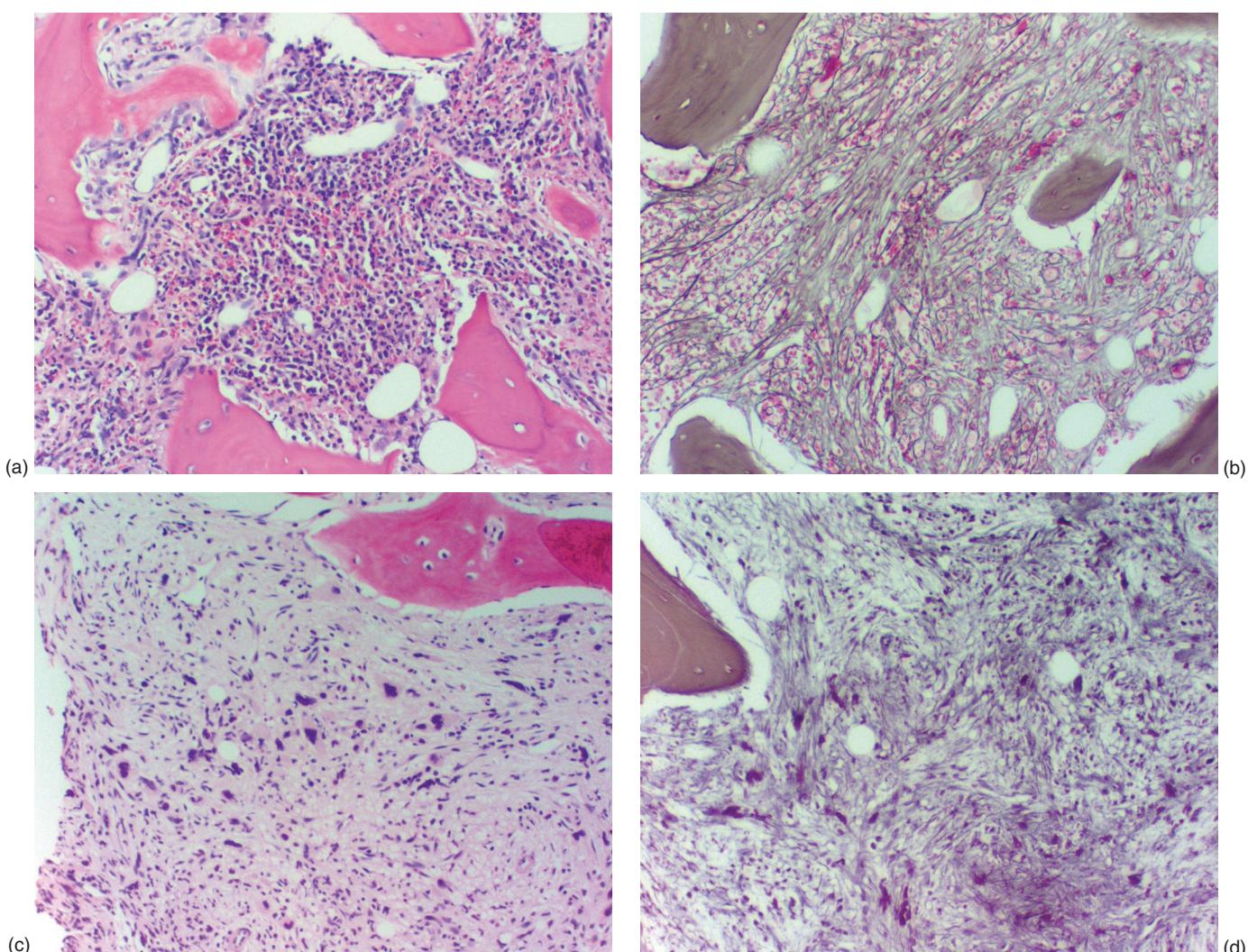


Figure 16-6 Bone marrow histopathologic findings in various stages of chronic idiopathic myelofibrosis. (a) The early fibrotic, cellular stage is characterized by hypercellularity and myeloid hyperplasia (hematoxylin-eosin stain [H&E]; original magnification, 85 \times); with (b) variable reticulin fibrosis (reticulin stain; magnification, 85 \times). (c) The later fibrotic stage is associated with extensive collagen deposition and loss of hematopoietic precursors, except for occasional residual megakaryocytes (H&E stain; magnification, 85 \times); (d) with more extensive reticulin fibrosis (reticulin stain; magnification, 85 \times). Photos courtesy of Steven J. Kussick, MD, PhD, University of Washington, Seattle, WA.

that takes into account changes in the risk profile brought about by acquisition of other disease factors during the course of the disease was established and designated the Dynamic International Prognostic Scoring System (DIPSS). The same factors were considered in both scoring systems except that hemoglobin <10 g/dL was given a higher score (score = 2) compared with other risk factors in the DIPSS. An updated version of this prognostic scoring system called DIPSS-Plus takes into account transfusion dependence, platelet counts <100 \times 10⁹/L, and the presence of certain chromosomal defects. Among 116 younger PMF patients, a hemoglobin <10 g/dL, the presence of constitutional symptoms, and circulating blasts >1% were independent adverse predictors and should be used to determine whether younger

patients undergo evaluation for allogeneic SCT. In that cohort, patients with 0 or 1 adverse factor had a median survival of 176 months, whereas patients with 2 or 3 adverse factors had a median survival of only 33 months. The presence of more adverse factors predicts for worse overall survival and increased risk for AML transformation (Table 16-7).

Therapy

General considerations

Conventional therapies for PMF are largely palliative and supportive; they do not alter the progression of marrow fibrosis and do not prolong survival. Asymptomatic PMF

Table 16-18 The three prognostic scoring systems currently used in primary myelofibrosis.

Risk factor	IPSS (No. of points)	HR (95% CI)	DIPSS (No. of points)	HR (95% CI)	DIPSS-Plus (No. of points)	HR (95% CI)
Age >65 years	1	1.95 (1.61-2.36)	1	1.98 (1.52-2.60)	DIPSS low = 0	—
Constitutional symptoms*	1	1.97 (1.62-2.40)	1	2.06 (1.61-2.65)	DIPSS Int-1 = 1	1.9 (1.2-3.1)
Hemoglobin <10 g/dL	1	2.89 (2.46-3.61)	2	4.18 (3.03-5.78)	DIPSS Int-2 = 2	3.6 (2.1-6)
WBC count >25 × 10 ⁹ /L	1	2.40 (1.83-3.14)	1	1.33 (1.33-2.29)		
Blood blasts >1%	1	1.80 (1.50-2.17)	1	1.82 (1.39-2.4)	DIPSS-high = 3	7.3 (4-13.3)
RBC transfusion dependence	—	—	—	—	1	1.4 (1.1-2)
Thrombocytopenia (<100 × 10 ⁹ /L)	—	—	—	—	1	1.6 (1.2-2.2)
Unfavorable karyotype†	—	—	—	—	1	2.4 (1.7-3.4)
Risk group	No. of factors	Median Survival (years)	No. of factors	Median Survival (years)	No. of factors	Median Survival (years)
Low	0	11.3	0	NR	0	15.4
Intermediate-1	1	7.9	1-2	14.2	1	6.5
Intermediate-2	2	44	3-4	4	2-3	2.9
High	>3	2.3	5-6	1.5	4-6	1.3

* 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.

NR = not reached

DIPSS = Dynamic International Prognostic Scoring System; CI = confidence interval; HR = hazard ratio; IPSS = International Prognostic Scoring System; RBC = red blood cell; WBC = white blood cell.

Data from Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. *Blood*. 2009;113:2895-2901; Passamonti F, Cervantes F, Vannucchi AM, et al.

A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). *Blood*. 2010;115(9):1703-1708; Gangat N, Caramazza D, Vaidya R, et al. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. *J Clin Oncol*. 2011;29(4):392-397.

patients without adverse prognostic features, with no or mild splenomegaly, and with no or mild leukocytosis or thrombocytosis can be observed without treatment. If constitutional or hypercatabolic symptoms develop, spleen size increases, or progressive elevations occur in the WBC count or platelet count, hydroxyurea is the treatment of choice. Busulfan, 6-mercaptopurine, and 2-chlorodeoxyadenosine (cladribine) have been used to ameliorate proliferative disease complications. IFN α can control blood counts and splenomegaly during the proliferative stage of disease in up to 50% of cases but is poorly tolerated by most patients given their preexisting constitutional symptoms. It is the drug of choice for the rare symptomatic young woman with PMF who requires treatment but is considering pregnancy. Anagrelide can be useful to control thrombocytosis after splenectomy or after a thromboembolic complication.

Anemia

Anemia may respond to high-dose recombinant EPO in up to 50% of cases; therapeutic success is most common in patients

with endogenous EPO levels <120 U/L who have not yet progressed to transfusion dependence. One study noted an increased risk of leukemia associated with EPO therapy; this has not been evaluated or confirmed in larger epidemiologic studies. Anemia, thrombocytopenia, and, less commonly, splenomegaly improve in up to one-half of patients who take low-dose thalidomide (ie, 50 mg/d), with or without a tapering-dose schedule of oral prednisone. Recent studies with pomalidomide therapy at 0.5 or 2 mg/d with an abbreviated course of prednisone resulted in improvement of anemia in 30%-40% of patients, with a substantial proportion of responders becoming transfusion independent. A minority of patients respond to androgens, including oxymetholone, nandrolone, and testosterone enanthate. Occasionally, patients with PMF-associated hemolytic anemia respond to corticosteroids, danazol, or cyclophosphamide.

Splenomegaly

Massive splenomegaly may cause portal hypertension and can be a major cause of morbidity in PMF because of pain,

anemia, and thrombocytopenia. Splenectomy is indicated for palliation of portal hypertension, refractory anemia, and symptoms not controlled by cytotoxic agents. With the conventional laparotomy approach in experienced centers, postoperative mortality is approximately 10%, and the median survival is approximately 1-2 years, although patients without poor prognostic features of the underlying disease may have much longer survival. Patients with laboratory evidence of disseminated intravascular coagulopathy appear to be at highest risk of thrombohemorrhagic complications during and immediately after splenectomy. Laparoscopic splenectomy can be performed in selected cases by an experienced surgeon. Splenectomy significantly increases the hematocrit in 30% of anemic patients. By contrast, severe thrombocytopenia (ie, $<20 \times 10^9/L$) is unlikely to improve after splenectomy. Rebound thrombocytosis (ie, $>600 \times 10^9/L$) and massive hepatomegaly are potential complications of splenectomy in patients with PMF. The increased platelet count in this setting can lead to life-threatening thrombotic or hemorrhagic events. Patients at increased risk of thrombocytosis are those with preoperative platelet counts $>50 \times 10^9/L$ to $100 \times 10^9/L$; 18%-50% of such patients will achieve postoperative levels $>600 \times 10^9/L$. In this setting, hydroxyurea or anagrelide should be started or the dose should be increased the moment the postoperative platelet count exceeds the normal range. Massive hepatomegaly resulting from accelerated extramedullary hematopoiesis develops in 16%-24% of patients after splenectomy. This complication may be difficult to control but may respond to cladribine. Some recent studies, but not others, have suggested an increased incidence of transformation to AML among splenectomized patients with PMF. The interval from diagnosis to surgery, the duration of follow-up, and the median survival among splenectomized patients differed among these studies, and this may account for the discordant observations. On a practical level, splenectomy should not be withheld in cases with advanced disease because of a concern of subsequent AML transformation.

For patients with severe splenomegaly who are not surgical candidates because of comorbidities, low-dose splenic irradiation (ie, 1-5 Gy delivered over 5-10 fractions) can be administered. Benefits may last several months, but irradiation carries a substantial risk of severe, prolonged cytopenias, which cannot be predicted by baseline blood counts or by the dose of radiation. Therefore, the risks and benefits of splenic radiotherapy should be considered carefully and discussed for patients with known limited hematopoietic reserve. Nevertheless, irradiation can effectively palliate pain in >90% of patients, and treatment can be repeated if necessary. Local irradiation can be effective for palliation of painful or threatening focal areas of extramedullary hematopoiesis (eg, with spinal or retroperitoneal myeloid tumors). With the

current availability of JAK inhibitors and its efficacy in reducing spleen volume and alleviating splenomegaly-related symptoms, there may be a change in the practice patterns of performing operative splenectomy and splenic irradiation.

JAK inhibitors

The high frequency of *JAK2* mutations in MPN patients, including in MF, became the basis for the development of novel JAK inhibitors. The first of its class, ruxolitinib is a potent and selective inhibitor of JAK1 and JAK2. Now approved by the FDA, it is the first pharmacologic agent to be FDA approved in MF. The pivotal phase III, multicenter, double-blind, placebo-controlled, randomized controlled 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 $\geq 50\%$ at 24 weeks were better in the ruxolitinib-treated patients (45.9%) compared with those receiving placebo (5.3%). A European counterpart also was conducted called COMFORT-II, which compared ruxolitinib versus best available therapy (BAT). In COMFORT-II, a spleen volume reduction of at least 35% was noted 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. Emerging data show that patients with MF treated with ruxolitinib who have high-risk disease and have $\geq 50\%$ reduction in spleen size have better overall survival compared with matched historical controls. A single-institution, sponsor-independent analysis of 51 patients enrolled on the phase I/II trial of ruxolitinib found no difference in survival rates between patients treated with ruxolitinib compared with their institution's 410 patients with PMF who were treated with standard therapy. In the COMFORT-II study, no significant difference in survival was found between patients treated with ruxolitinib and patients treated with BAT, although this trial was not powered to detect a statistically significant effect on overall survival. Of note, patients who are *JAK2V617F* positive or negative respond equally to ruxolitinib, such that mutational testing is not required to guide the use of JAK inhibitor therapy. The main side effects of ruxolitinib are reversible, dose-dependent anemia and thrombocytopenia, and care must be taken in using these agents in patients with preexisting anemia or thrombocytopenia. Other JAK inhibitors are in various stages of clinical trial investigation, including SB1518, CYT387, SAR302503, LY2784544, and many others.

Stem cell transplantation

In younger individuals with PMF with poor prognostic features, allogeneic hematopoietic SCT should be considered because it represents the only potentially curative treatment. Studies using standard ablative conditioning regimens demonstrated that a subset of patients achieve long-term clinical and molecular remission after SCT. Results using unrelated donors were equivalent to those with HLA-matched sibling transplantations. An early multi-institutional experience, including patients <55 years old receiving marrow or peripheral blood stem cells predominantly from related donors, demonstrated a 47% 5-year probability of survival and a 40% rate of histologic and hematologic remission. Previously splenectomized patients engraft more rapidly after SCT; however, pretransplantation splenectomy is not recommended for this indication alone. Severe fibrosis may correlate with delayed engraftment and higher posttransplantation mortality in some patients but is not associated with graft failure. Responding patients had resolution of marrow fibrosis over months to years, and splenomegaly regressed to normal or minimally palpable in >90%. Inferior transplantation outcome was associated with higher pretransplantation disease risk features, high-grade marrow fibrosis, older age, and the presence of a marrow cytogenetic abnormality. On the basis of these recent data, a myeloablative allogeneic transplantation should be considered when poor prognostic features develop in children and adults with PMF up to 55 years of age who have an HLA-identical donor. The indications for transplantation at an earlier stage of disease, when the prognosis and survival are more favorable and less predictable, remain the subject of debate. Alternative transplantations approaches for PMF include allogeneic nonmyeloablative SCT, which may be the best transplantation option for patients 55–70 years of age. In a recent study of 21 patients (age 27–68 years), reduced-intensity conditioning resulted in long-term disease-free survival in the majority of patients with acceptable toxicities. Moreover, patients treated with reduced-intensity conditioning regimens can achieve stable donor chimerism, significant improvement in blood counts, and significant decrease in marrow fibrosis. Although SCT represents an important therapy for high-risk patients with a matched donor, acceptable comorbidities, and age <70, the majority of patients with PMF are not candidates for SCT; thus, new therapies are needed for the majority of patients with PMF.

Key points

- More than two-thirds of patients with PMF are diagnosed at age 60 years or older, and their median survival time is only 3.5–5.5 years.

Key points (continued)

- PMF must be differentiated from advanced PV, ET, CML, acute megakaryoblastic leukemia, myelodysplasia with fibrosis, infiltrative malignant processes, and infectious or autoimmune diseases associated with reactive marrow fibrosis.
- The majority of patients with PMF develop anemia, splenomegaly, and hypercatabolic symptoms during the course of their disease; anemia (hemoglobin <10 g/dL) and a high or low WBC count (>30 or <4 × 10⁹/L) predict a shorter survival.
- Therapeutic approaches to PMF are guided by the presence of specific symptoms and disease-related complications.
- Splenectomy should be considered for palliation of symptomatic massive splenomegaly; however, postsplenectomy complications include perioperative mortality, rebound thrombocytosis, and massive hepatomegaly.
- Myeloablative allogeneic SCT should be considered for patients <55 years of age with poor prognostic features who have an HLA-compatible donor. Nonmyeloablative allogeneic SCT may benefit selected patients who are older or who are not candidates for conventional SCT.

Myeloproliferative neoplasm, unclassifiable

The term MPN, unclassifiable (MPN-U) is a newly defined WHO entity, and it 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 a *BCR-ABL1* fusion or the *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangements, excludes the diagnosis of MPN-U.

Epidemiology

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 can be quite variable. 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.

Myeloid (and lymphoid) neoplasms associated with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*

The revised 2008 WHO classification has recently recognized these three rare conditions as a new category of myeloid–lymphoid neoplasms associated with marked and persistent eosinophilia ($\geq 1.5\text{--}3 \times 10^9/\text{L}$) and chromosomal rearrangements, leading to constitutive activation of the *PDGFRA*/ *PDGFRB* or *FGFR1* genes. 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 mesylate therapy. All three disorders can present with classic features of MPNs; however, it is still not clearly established whether *PDGFRB*-related rearrangements can manifest as lymphoid neoplasms.

Myeloid and lymphoid neoplasms associated with *PDGFRA* rearrangement

Clinical case

A 52-year-old mechanic suffered a stroke about 6 months earlier. No definitive etiology was identified, but he suffered from left-sided residual weakness from the event. In the past 3 months, he started to have recurrent headaches, rhinorrhea, wheezing, weight loss of 15 pounds, diarrhea, night sweats, pruritus, and lower-extremity edema. He underwent a routine blood test, including a CBC, which showed the following: WBC = $15 \times 10^9/\text{L}$, Hgb = 10.3 g/dL, MCV = 89 fL, platelets = $224 \times 10^9/\text{L}$. Percent differentials of WBC are as follows: neutrophils 67%, lymphocytes = 12%, monocytes = 3%, eosinophils = 18%, basophils = 0%. The corresponding absolute counts of WBC differentials are as follows: ANC = $10 \times 10^9/\text{L}$, absolute lymphocyte count = 1.8×10^9 , absolute monocyte count (AMC) = $0.4 \times 10^9/\text{L}$, and AEC = $2.7 \times 10^9/\text{L}$. There were no circulating blasts in the peripheral blood. His complete metabolic panel showed some mild hyponatremia, and the rest of the results were unremarkable. Workup for an underlying connective tissue disease, other neoplastic process, and parasitic infection were negative. A CT scan of the sinus just revealed thickening of the right sphenoid sinus. Total IgE is elevated (IgE = 283 kU/L). CT of the chest showed patchy opacities consistent with (c/w) bronchiolitis or vasculitis. CT scan of the abdomen

Clinical case (continued)

and pelvis showed a hyperdense right renal mass, which was first appreciated 3 years prior and stable in size and splenomegaly. FISH for BCR-ABL was negative. Transthoracic echocardiography showed a diminished ejection fraction of 30% and the presence of restrictive cardiomyopathy. FISH for the CHIC2 deletion was positive, a surrogate for the *FIP1L1-PDGFR* fusion.

PDGFRA is a member of the family of class III receptor TKs, which also includes *PDGFRB*, c-KIT, and FLT3. 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*. Although most cases of *PDGFRA*-related neoplasms present with clinical features of CEL with prominent involvement of mast cells, they also may present with features of AML or precursor T-cell lymphoblastic lymphoma. Furthermore, several other partner genes have been implicated in the pathogenesis of *PDGFRA*-related neoplasms, including *BCR*, *ETV6*, *KIF5B*, and *CDK5RAP2*.

Epidemiology

Although the true incidence of *PDGFRA*-related neoplasms is not really 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%–10% of patients in industrial countries who present with idiopathic hypereosinophilia can be found to have the *FIP1L1-PDGFR* fusion.

Pathobiology

The classic *PDGFRA*-related chromosomal rearrangement, the *FIP1L1-PDGFR* fusion gene, is generated by an 800-kilobase interstitial deletion on chromosome 4q12. This cryptic deletion, when using standard cytogenetic banding techniques, explains why most cases of CEL apparently have a normal karyotype. Expression of *FIP1L1-PDGFR* transformed a murine hematopoietic cell line and was constitutively active in these cells and led to increased STAT5 phosphorylation. Similar transforming properties were noted when *STRN-PDGFR* or *ETV6-PDGFR* fusion genes were transfected into murine hematopoietic cell lines.

Clinical features

PDGFRA-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,

angioedema, 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*-related neoplasms is endomyocardial fibrosis with ensuing restrictive cardiomyopathy. Elevated serum tryptase of >12 ng/mL also usually are present.

Diagnostic criteria

The revised 2008 WHO classification defines *PDGFRA*-related neoplasms as MPNs with prominent eosinophilia and the presence of the *FIP1L1-PDGFRA* fusion gene. Thus, the most prominent diagnostic feature of patients with *PDGFRA*-related neoplasms is the presence of peripheral blood mature eosinophilia. Anemia and thrombocytopenia occasionally are present. Bone marrow biopsy demonstrates marked hypercellularity with increased mature and precursor eosinophils and an increased number of mast cells. Immunophenotyping is typical for activated eosinophils with expression of CD23, CD25, and CD69. Mast cells usually are double negative for CD2 and CD25. The gold standard for the diagnosis of these neoplasms is demonstration of the fusion gene. As mentioned, most cases of CEL present with normal karyotype; thus, FISH and 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-PDGFRA* fusion gene. RT-PCR can be used both for diagnosis and monitoring of disease response and for minimal residual disease monitoring.

Treatment

As of 2009, the mainstay of therapy for patients with *PDGFRA*-related neoplasms is the use of imatinib mesylate. Since initially reported in 2001, 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 mesylate (100-400 mg/d). In two of these studies, the European LeukemiaNet 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 *FIP1L1-PDGFRA* fusion transcripts, and 9 of 11 patients achieved a complete molecular remission. Similarly, an Italian multicenter study demonstrated high levels of durable (median, 251 months) complete molecular remissions in 27 patients with *PDGFRA*-related neoplasms. Unfortunately, it appears that withdrawal of imatinib therapy

is followed by a rapid increase in *FIP1L1-PDGFRA* transcript levels. Although less common than in *BCR-ABL*-positive CML, kinase domain mutations that confer resistance to imatinib therapy can occur in *FIP1L1-PDGFRA* rearrangement-positive myeloid or lymphoid neoplasms, and includes T674I and D842V. Other TK inhibitors have been used in this setting with only modest and transient benefit.

Course and prognosis

In the preimatinib era, the prognosis of patients with HES was poor; the median survival time was 9 months, and the 3-year survival was only 12%. Patients generally had advanced disease, with congestive heart failure accounting for 65% of the identified causes of death. More recently, an observed 5-year survival rate of 80%, decreasing to 42% at 15 years, was noted. Since the recognition that *PDGFRA*-related neoplasms are highly sensitive to TK inhibitors, most patients achieve and remain in complete hematologic and molecular remission within a few weeks of initiation of therapy.

Myeloid neoplasms associated with *PDGFRB* rearrangement

Clinical case

A 66-year-old retired computer software analyst underwent a general check-up, which is part of his yearly routine since retiring 4 years prior. He is feeling well and has no complaints. His workup, which included a lipid profile and complete metabolic panel, were unremarkable. He was notified, however, of the presence of abnormalities in his CBC. His CBC showed WBC = $24 \times 10^9/L$, Hgb = 11.0 g/dL, MCV = 81 fl, platelets = $324 \times 10^9/L$, ANC = $16 \times 10^9/L$, absolute lymphocyte count = $2.5 \times 10^9/L$, AMC = $3 \times 10^9/L$, and AEC = $2.7 \times 10^9/L$. There was 1% circulating peripheral blood blasts. Review of a prior CBC dating back to 8 months prior showed leukocytosis (WBC = $19 \times 10^9/L$) with neutrophilia (ANC = $14 \times 10^9/L$), absolute moncytosis (AMC = $1.5 \times 10^9/L$) and absolute eosinophilia (AEC = $2.6 \times 10^9/L$). He was referred to a hematologist who noted the prior findings and reviewed a peripheral blood smear, which showed dysplastic changes in some of the neutrophils. A bone marrow aspiration and biopsy was recommended, which he underwent 3 days later. The bone marrow showed 2% bone marrow blasts in the aspirate with increased numbers of monocytes. Dysplastic-looking neutrophilic precursors and monocytes also were appreciated. The core biopsy showed a hypercellular bone marrow (80%) with mild reticulin fibrosis. Metaphase cytogenetics showed t(5;12)(q31~33;p12).

PDGFRB-related neoplasms are a distinct group of myeloid neoplasms associated with rearrangement of the

PDGFRB gene, located on the long arm of chromosome 5 (5q31-33). Patients with *PDGFRB*-related neoplasms tend to present with features characteristic of chronic myelomonocytic leukemia with associated eosinophilia. In 1994, Golub et al. (1994) were the first to characterize the t(5;12)(q31-q33;p13) translocation involving *ETV6*(12p13) and *PDGFRB* (5q33). Since then, >20 partner genes have been identified to collaborate in the development of *PDGFRB*-related neoplasms.

Epidemiology

Similarly to its counterpart, *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. *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.

Clinical features

PDGFRB-related neoplasms are also systemic disorders with extensive involvement of peripheral blood and bone marrow. Extramedullary manifestations, such as skin infiltration and splenomegaly, are common.

Diagnostic criteria

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 fusion gene. Peripheral blood usually presents with leukocytosis (neutrophilia or monocytosis) along with anemia and thrombocytopenia. Bone marrow is hypercellular, and mast cells can be increased in number.

Treatment

Imatinib mesylate is also the mainstay of therapy for patients with *PDGFRB*-related neoplasms. Data to support this recommendation are scarce. A recent phase II study from a German group demonstrated that complete molecular remissions were achieved in 3 of 5 patients with *PDGFRB*-related neoplasms after 3-18 months of low to conventional doses of imatinib. Another multicenter study reports on the outcomes for 12 patients with *PDGFRB*-related neoplasms

who received imatinib therapy for a median of 47 months. Eleven patients had prompt responses with normalization of peripheral blood cell counts and disappearance of eosinophilia; 10 had complete resolution of cytogenetic abnormalities and decrease or disappearance of fusion transcripts as measured by RT-PCR. Thus, it also appears that TK inhibitors positively affect the outcome of patients with *PDGFRB*-related neoplasms.

Course and prognosis

Prognosis in the preimatinib era was very poor for patients with *PDGFRB*-related neoplasms; the median survival time did not exceed 2 years. Although there are only small case series on the impact of imatinib on the survival of patients with *PDGFRB*-related neoplasms, these studies suggest a similar benefit to those seen in patients with *PDGFRA*-related neoplasms, with median survivals exceeding 5 years. Better recognition of these disorders and earlier initiation of therapy with TK inhibitors undoubtedly will continue to improve the outcome of these patients.

Myeloid and lymphoid neoplasms associated with *FGFR1* abnormalities

The revised 2008 WHO classification recognizes, for the first time, this extremely uncommon and heterogeneous group of neoplasms that 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.

Epidemiology

FGFR1-related neoplasms have been reported across a wide age range (3-84 years), and the median age of diagnosis is 32 years. Females constitute approximately 40% of the cases.

Pathobiology

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 TKD of *FGFR1*. These fusion genes (*ZNF198-FGFR1*), 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.

Clinical features

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

Diagnostic criteria established by the 2008 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. More than 10 fusion gene partners so far are described in *FGFR1* rearrangement neoplasms, including *CEP110*, *FGFR1OP1*, *FGFR1OP2*, *TRIM 24*, *MYO18A*, *HERVK*, and *BCR*.

Treatment

Early intensive therapy followed by allogeneic SCT remains the only potential curative therapy for patients with *FGFR1*-related neoplasms. Interestingly, PKC412 has demonstrated in vitro activity against one subtype of the *FGFR1* fusion gene.

Course and prognosis

The prognosis for patients with *FGFR1*-related neoplasms is very poor with evolution to AML typically occurring within 1-2 years. The clinical aggressiveness and diminished awareness about the features of this entity and the lack of approved TK inhibitors make the management of these patients very challenging.

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Acquired marrow failure syndromes: aplastic anemia, paroxysmal nocturnal hemoglobinuria, and myelodysplastic syndromes

Phillip Scheinberg and David P. Steensma

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CHAPTER
17



Acquired marrow failure syndromes: aplastic anemia, paroxysmal nocturnal hemoglobinuria, and myelodysplastic syndromes

Phillip Scheinberg and David P. Steensma

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Introduction

Bone marrow failure refers to the inability of hematopoiesis to meet physiologic demands for the production of healthy blood cells. Pancytopenia may result from marrow failure, or cytopenias involving a single myeloid lineage (eg, anemia) may dominate; usually lymphopoiesis is relatively preserved.

The causes of marrow failure are diverse and may either be extrinsic to the marrow, as in the disordered immune response that characterizes aplastic anemia, or intrinsic, as in the hematopoietic progenitor or stem cell defects that underlie the myelodysplastic syndromes (MDSs). Bone marrow failure 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 reviews acquired marrow failure syndromes, including aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), and MDS. For discussion of inherited marrow failure syndromes, please refer to Chapter 15.

Aplastic anemia

Definition

AA is a hematopoietic stem cell (HSC) disorder associated with markedly reduced marrow cellularity and deficient blood cell production. Classification and prognosis in AA are related to the severity in the depression of peripheral blood counts. *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

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off-label drug use: Dr. Steensma: Epoetin, darbepoetin, filgrastim, sargramostim, and romiplostim in myelodysplastic syndromes. Dr. Scheinberg: Alemtuzumab, eltrombopag, and high-dose cyclophosphamide in aplastic anemia; androgenic steroids in paroxysmal nocturnal hemoglobinuria.

Table 17-1 Classification of aplastic anemia by severity.

Severe aplastic anemia*	Moderate aplastic anemia
Bone marrow cellularity <30%	Decreased bone marrow cellularity
Depression of at least two of the following three hematopoietic lineages: Absolute neutrophil count $<0.5 \times 10^9/L$	Depression of at least two of three hematopoietic lineages not fulfilling the severity criteria as specified in the left column
Transfusion dependence, with absolute reticulocyte count $<60 \times 10^9/L$ or platelet count $<20 \times 10^9/L$	

* Very severe aplastic anemia is reserved for patients who fulfill criteria for severe AA but with an absolute neutrophil count $<0.2 \times 10^9/L$.

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 17-1). AA is associated with normal cytogenetics. An abnormal karyotype in a patient with a hypocellular marrow is more consistent with a diagnosis of MDS, although some investigators believe that certain chromosomal abnormalities such as trisomy 8 or deletion 13q can still be consistent with an AA diagnosis.

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, although 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 recent history of cytotoxic chemotherapy or exposure to ionizing radiation.

Epidemiology, etiology, and pathogenesis

AA is rare in Western Europe and the United States (2 cases per million population per year), but the incidence in China, Southeast Asia, and Mexico is estimated to be three to four times higher. AA is primarily a disease of children and younger adults, with another peak in incidence in patients 60 years and older; in the latter group, some cases may reflect diagnostic overlap with hypoplastic MDS.

AA can arise during pregnancy or in association with hepatitis. Hepatitis-associated AA accounts for 2%-5% of cases of AA in Europe and 4%-10% of cases in East Asia. AA has been reported to occur in 28%-33% of patients requiring orthotopic 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 known hepatitis viruses and often is referred to as *hepatitis/AA syndrome*. AA evolves with a typical delay of several weeks to months after the episode of hepatitis, usually after improvement of liver enzymes.

AA can be acquired or constitutional. Idiopathic and acquired AA is perceived as a T-cell-mediated autoimmune process that accounts for most cases in North America being more common

than AA associated with medication exposure, pregnancy, or hepatitis (Table 17-2). The association of AA with certain drugs like chloramphenicol has been reported with some drug-induced AA models developed with this antibiotic, which have not fully recapitulated the human syndrome. Many other drugs have been associated with AA; the link with some drugs has been doubtful, whereas with others, the evidence is more convincing. The medications indomethacin, diclofenac, butazones (such as phenylbutazone), clopidogrel, antithyroid medications (such as propylthiouracil), and gold salts are more clearly associated with development of AA.

Irrespective of the etiology, hematopoiesis is reduced markedly in all patients with AA, as reflected by marrow histology, low numbers of circulating or marrow CD34 cells, diminished numbers of long-term culture-initiating cells (a surrogate measure of HSCs), and poor hematopoietic colony formation in cells obtained from an aplastic marrow. Obligatory involvement of multiple lineages points toward HSCs or very early hematopoietic progenitor cells as main targets of the pathophysiologic mechanism in AA (Figure 17-1).

Clinical response to immunosuppressive therapy targeting T-cells (eg, antithymocyte globulin [ATG]), described further

Table 17-2 Classification of aplastic anemia by etiology.

Acquired aplastic anemia
<i>Primary</i>
Idiopathic aplastic anemia
Pregnancy-associated aplastic anemia
Aplastic anemia/paroxysmal nocturnal hemoglobinuria syndrome (AA/PNH)
<i>Secondary</i>
Drug associated
Iatrogenic/cytotoxic
Idiosyncratic
Radiation associated
Iatrogenic
Accidental
Viruses
Epstein-Barr virus
Cytomegalovirus
Hepatitis/aplastic anemia syndrome (seronegative for hepatitis viruses)
Pancytopenia of autoimmune diseases

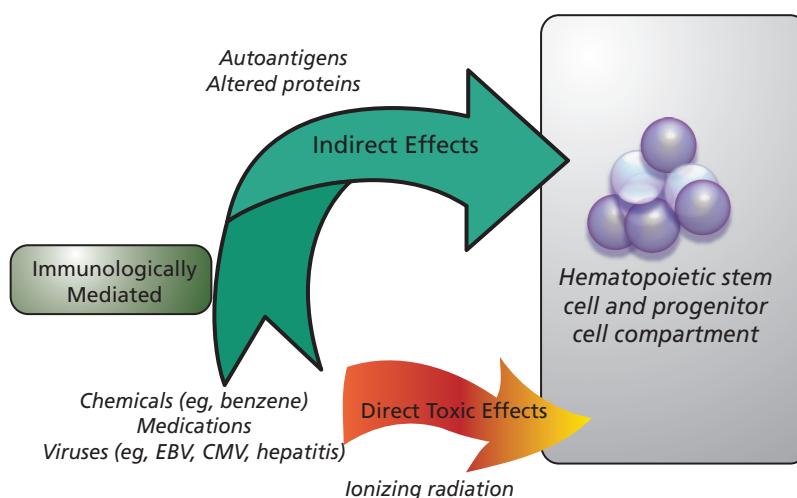


Figure 17-1 Types of stem cell injury in aplastic anemia. Aplastic anemia can result both from direct toxic effects on hematopoietic stem cells and progenitors and from an aberrant T-cell–driven immune response. CMV = cytomegalovirus; EBV = Epstein-Barr virus.

below in the “Immunosuppressive therapy” section, supports an immune-mediated pathogenesis of AA. Activated cytotoxic T-cells produce interferon- α and tumor necrosis factor α (TNF α), which suppress hematopoiesis. In addition, Fas receptor (CD95) expression by hematopoietic progenitor and stem cells is increased in patients with AA and likely is induced by interferon- α and TNF α . Ligand binding to the Fas death receptor on the surface of hematopoietic progenitors could contribute to marrow aplasia by triggering apoptosis. Inhibitory cytokines also exert a direct inhibitory effect on hematopoietic progenitors. Although the T-cell–mediated process is intrinsically polyclonal, oligoclonal expansion of CD8 $^{+}$ T cells has been observed in some AA patients, raising the possibility that these might represent immunodominant autoreactive clones. Human leukocyte antigen (HLA)-DR15, a split of HLA-DR2, is overrepresented in AA (40%-50% of patients, compared with an antigen frequency of ~20% in the general population), which also suggests an immune pathogenesis of idiopathic AA. A decrease in regulatory T-cells (T_{reg}) and an increase in interleukin-17-producing T-helper cells (T_h17 cells) have been described at diagnosis in AA, with the ratio between these two T-cell subsets normalizing in responding patients to immunosuppression. Many of these in vitro observations have been confirmed in a murine AA model, corroborating with an autoimmune pathogenesis. Although diverse triggers, such as viruses or chemical haptens, may serve as inciting events in individual cases, the final autoimmune pathway appears to be uniform.

Recent data also implicate intrinsic HSC defects in some AA cases. Mutations in genes encoding components of telomerase, *TERC* and *TERT*, have been described in patients who lack the overt clinical stigmata of DC (a congenital disorder associated with germline *TERC* and *TERT* mutations and, more commonly, mutations in *DKC1* [encoding dyskerin]). Telomeres progressively shorten over successive cell divisions and the telomerase complex repairs the naturally occurring erosion of chromosome ends, avoiding replicative senescence. This complex consists of an RNA template (encoded by

TERC), a catalytic subunit (encoded by *TERT*), and associated proteins, which adds short nucleotide repeats (TTAGGG) to the ends of chromosomes (telomeres) during each cellular division. When a critically short telomere length is reached, cell death ensues. Although telomerase expression is generally low in most somatic cells, expression in lymphocytes and HSCs is high, in view of their life-long high replicative capacity. Telomerase dysfunction is associated with accelerated telomere shortening, and may result in premature death of rapidly proliferating cells (eg, HSCs). Thus, it is possible that the inability to properly counter telomeric attrition may contribute to AA pathogenesis in some patients, because of a lower capacity to restore hematopoiesis. It is unclear whether a *TERC* or *TERT* genetic defect is sufficient to define the pathogenesis in some cases of apparent acquired AA, or whether it is a contributor to other pathophysiologic mechanisms of marrow destruction, such as an aberrant immune system.

Clinical presentation

Symptoms of AA are a consequence of lack of production of blood cells. Patients present with pallor and fatigue due to anemia, with mucocutaneous bleeding due to thrombocytopenia, or with infection due to neutropenia. More severe hemorrhage in the central nervous system or gastrointestinal tract is not typical on disease presentation. AA usually 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, exocrine pancreatic insufficiency, or other congenital anomalies may suggest an inherited bone marrow failure state (see Chapter 15). 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 query in the medical interview about earlier blood count abnormalities, macrocytosis, or relevant pulmonary (fibrosis) or liver disease (cirrhosis) in the patient or the patient's family, which are frequent in telomere disorders.

Malignant or markedly dysplastic cells are not seen in the marrow in AA, although mild dysplastic features sometimes are noted, which can cause diagnostic confusion with hypoplastic MDS. In the marrow, all myeloid hematopoietic cell lines are diminished, whereas residual lymphocytes and plasma cells frequently are observed. The marrow is characteristically hypocellular with an expansion of the fat cells and no increase in reticulin.

Marrow biopsy is the gold standard for assessing marrow cellularity. Because residual marrow cellularity in AA may be patchy and variable, results should be interpreted within the clinical context of the patient. Cytogenetic results are typically

normal in AA, and a finding of an abnormal karyotype is suggestive of a dysplastic process. The identification of monosomy 7 in the karyotype at diagnosis strongly suggests MDS, as most of these cases are refractory to immunosuppression and carry a higher rate of progression to high-risk MDS.

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 17-3; Figure 17-2). The most common disorders include MDS, aleukemic leukemia, PNH, myelofibrosis, hairy cell leukemia, certain infections (tuberculosis), nutritional deficiency (eg, anorexia nervosa), T-cell large granular lymphocyte (T-LGL) disease (T-LGL can coexist with AA), and HIV infection. The diagnostic approach to the patient with pancytopenia 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; bone marrow aspirate and biopsy with cytogenetic studies; and chromosome fragility tests, particularly in children and younger adults.

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

Pancytopenia

Hypocellular bone marrow

Primary marrow disorders

- Acquired aplastic anemia
- Constitutional forms of marrow failure (FA, DC)
- Hypocellular MDS
- Hairy cell leukemia
- Aleukemic leukemia

Rarely

- Lymphoma
- Myeloma
- Primary myelofibrosis

Systemic illnesses

- Hypothyroidism
- Anorexia nervosa
- Infections
- Tuberculosis
- Q fever

Hypercellular bone marrow

Direct marrow involvement

- Myelodysplastic syndrome
- Paroxysmal nocturnal hemoglobinuria
- Primary myelofibrosis
- Lymphoma
- Metastatic carcinomas and sarcomas

Systemic illnesses

- Systemic lupus erythematosus
- Hypersplenism
- Sepsis
- Alcoholism
- Brucellosis
- Ehrlichiosis
- Vitamin deficiencies (B12, folate)

Table 17-3 Differential diagnosis of idiopathic aplastic anemia and pancytopenia.

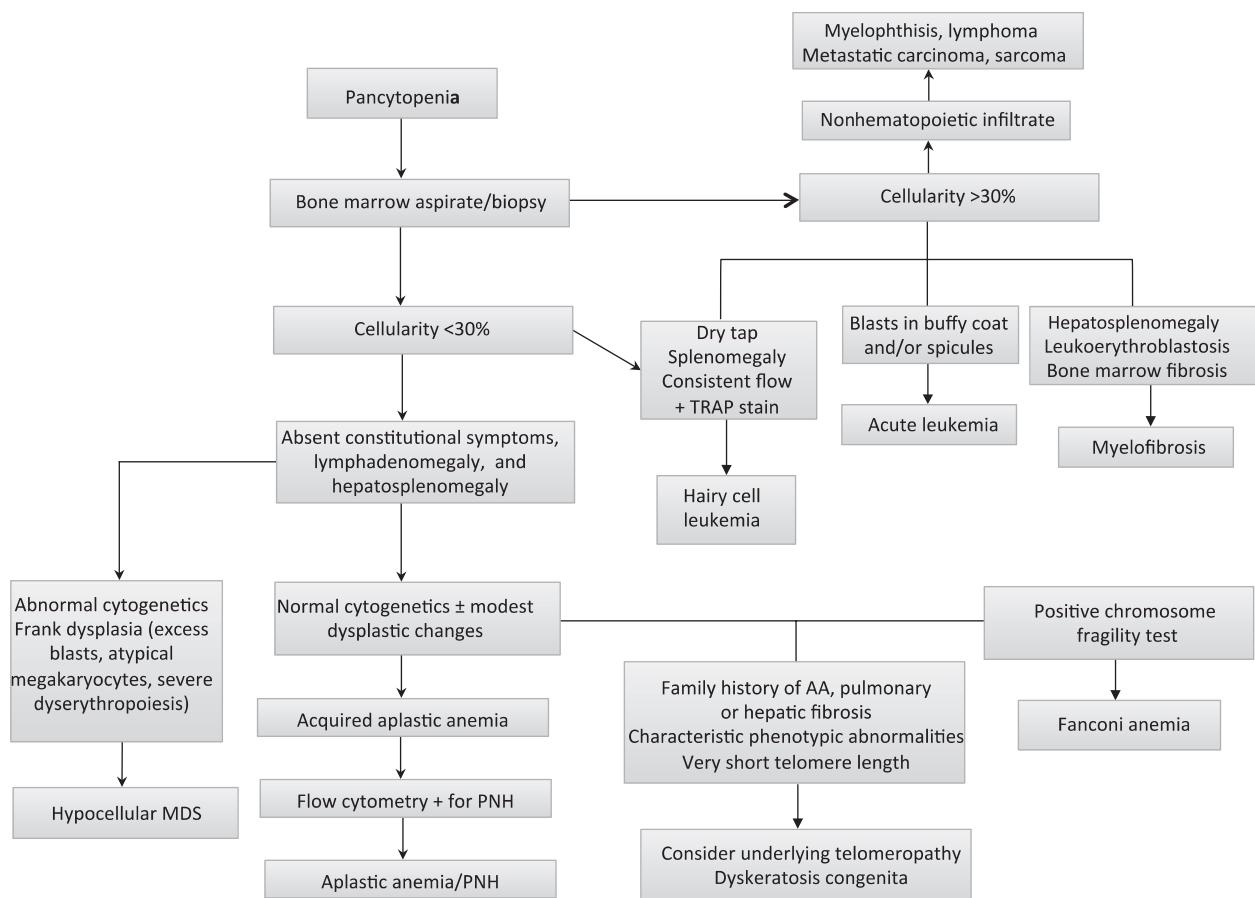


Figure 17-2 Diagnostic algorithm of primary marrow causes of pancytopenia. In patients with a hypocellular marrow, the main differential is between hypocellular myelodysplasia (MDS) and aplastic anemia (AA). Normal cytogenetics and only modest dysplastic changes favors AA, whereas more pronounced dysplasia (micromegakaryocytes, left shift myelopoiesis with increase in blasts, significant dyserythropoiesis) and an abnormal cytogenetics favors MDS. Patients with AA and a paroxysmal nocturnal hemoglobinuria (PNH) clone, are classified as AA/PNH. In those with normal or increased marrow cellularity, differential includes a myelophthitic process (lymphoma, metastatic carcinoma, or sarcomas), rare presentations of leukemia, myelofibrosis, and hairy cell leukemia. Close attention should be paid to the spicules, which may harbor a blast population indicating a leukemic process. Hepatosplenomegaly, increase in marrow reticulin (fibrosis) and leukoerythroblastic peripheral smear favors myelofibrosis. In hairy cell leukemia marrow cellularity is often elevated, but in 10%-20% of cases, cellularity can be low. A dry tap and splenomegaly in a pancytopenic patient hints toward the possibility of hairy cell leukemia. The diagnosis is confirmed by immunophenotype and a marrow that stains positive for tartrate resistant acid phosphatase (TRAP).

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 epiphrenomenon of the eating disorder, possibly 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 B12 or folate deficiency is typically hypercellular and megaloblastic rather than hypocellular. HIV infection or AIDS is associated with cytopenia, morphologic dysplasia, and marrow hypopcellularity in ~10% of cases. A careful inquiry into HIV risk factors and an HIV test are prudent.

T-LGL disease is a rare condition characterized by circulating T-cells bearing the CD57 marker of effector or cytotoxic

T-cells. T-LGL disease, like PNH, can coexist with AA or MDS. T-LGL disease should be considered if increased LGL are noted on the peripheral blood smear or if the patient has rheumatoid arthritis, which is known to be associated with T-LGL disease. Single-lineage cytopenia is more common in T-LGL with clinical presentation of isolated anemia or neutropenia most typical. Flow cytometry and testing for a clonal T-cell receptor gene rearrangement is appropriate when T-LGL is suspected.

A possible underlying cause of AA is FA, which can present with apparent acquired AA in adulthood without other classic features of this disease. Therefore, diepoxybutane (DEB) or mitomycin C testing to exclude chromosome fragility should be considered in patients with AA <40 years of age, even in the absence of musculoskeletal abnormalities.

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 ATG or cyclosporine immunosuppression (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 sometimes cytogenetically abnormal clones can be observed transiently in AA.

In acquired AA, PNH clones can be detected by flow cytometry in 40%-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.

Key points

- AA is a diagnosis of exclusion.
- Differential diagnosis for pancytopenia with hypocellular marrow includes the following:
 - Idiopathic AA
 - Constitutional forms of bone marrow failure
 - Hypoplastic MDS
 - Aleukemic leukemia
 - PNH
 - Myelofibrosis
 - Rheumatologic disorders (eg, systemic lupus erythematosus)
 - Hairy cell leukemia
 - Infections (eg, tuberculosis, histoplasmosis, HIV, Epstein-Barr virus, hepatitis)
 - Anorexia nervosa
 - T-LGL disease
 - Folate or vitamin B12 deficiency (marrow typically hypercellular)
 - Drugs or toxins
- Diagnostic approach to the patient with pancytopenia includes the following:
 - History, including medications, previous chemotherapy or radiation, occupational toxic exposures, HIV risk factors, family history

Key points (continued)

- Physical examination, paying particular attention to presence of organomegaly, lymphadenopathy, or congenital abnormalities
- Complete blood count, including reticulocyte count and peripheral smear examination
- Liver function tests
- Vitamin B12 and folate levels
- LDH, haptoglobin, and flow cytometry for PNH
- Bone marrow aspirate and biopsy
- Cytogenetic studies
- Chromosome fragility tests, in particular in children and younger adults <40 years of age

Therapy

Without treatment, almost all patients with severe 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 standard of care for moderate AA, in contrast, 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 AA can spontaneously recover normal hematopoiesis. Spontaneous remission is most often seen with drug-induced AA and usually occurs within 1-2 months of discontinuing the offending drug.

Therapy of severe AA consists of allogeneic hematopoietic stem cell transplantation (HSCT) or immunosuppressive therapy. At the time of diagnosis, all potential transplantation candidates should be HLA typed to identify a sibling donor or a potential unrelated donor. Because registries of unrelated donors frequently take several months to identify a donor, this process should be initiated immediately if a sibling donor cannot be identified, especially in younger patients.

Supportive care, transfusions, and hematopoietic growth factors

Supportive care is instituted to sustain blood counts and alleviate symptoms of pancytopenia. Potential marrow toxins should be withdrawn. Supportive therapy consists of transfusion of irradiated, leukocyte-depleted blood products because patients are at risk for alloimmunization from chronic transfusions. If the patient is cytomegalovirus (CMV) negative, it is best to use CMV-negative blood products or leukocyte-depleted products. Transfusions should not be withheld from symptomatic patients. In patients who are candidates for allogeneic HSCT, the use of leuko-depleted blood products is critical to decrease the risk of alloimmunization, and transfusions should never be given from family

members because doing so would increase the risk of subsequent graft rejection. Prophylactic transfusions to maintain platelets $>10 \times 10^9/L$ generally prevents major hemorrhagic complications. The role of preventive antibiotics in neutropenic patients is not well defined.

Most patients with AA have an elevated serum erythropoietin level and do not respond to recombinant erythropoietin. Although typical AA also will not respond to myeloid growth factors either (ie, granulocyte colony-stimulating factor [G-CSF] or granulocyte-macrophage colony-stimulating factor [GM-CSF]), some patients do respond, and these growth factors may have a role in decreasing infectious morbidity (either for primary or secondary prevention of infection) 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, an oral thrombopoiesis-stimulating agent, eltrombopag, produced improvement in blood counts in patients with severe AA who were refractory to at least one course of immunosuppression. These preliminary data suggest that eltrombopag could be an option in refractory cases. The use of eltrombopag continues to be investigated as “salvage” therapy, as well as in the upfront setting along with immunosuppression, to better define its role in AA.

Corticosteroids are ineffective, increase the risk of infection, and should not be used as therapy in AA. The role of corticosteroids in severe AA 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

- Use irradiated, leukocyte-depleted blood products.
- Transfusions should not be from family members (especially in transplant candidates).
- Growth factors may have a role in decreasing infectious morbidity while the patient awaits definitive treatment with immunosuppression or stem cell transplantation for severe AA.
- Typical AA does not 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

In AA, the pretransplantation conditioning regimen primarily is administered to provide immunosuppression, which enables the donor stem cells to engraft and also eliminates activated immune cells that may be causing the marrow aplasia. 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 10-15 years. This practice has resulted in an untoward consequence in transplanted AA patients, where several reports in the past 5 years from Europe and the United States have shown an increase rate of graft-versus-host disease (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.

The standard conditioning regimen in matched-sibling transplantation includes ATG and cyclophosphamide, which results in a long-term survival rate of about 75%-80% in patients of all ages and is superior to conditioning regimens based on total lymphoid irradiation. The latter conditioning regimen has been shown to result in a higher incidence of secondary malignancies, infertility, and, in children, retardation of growth and development.

A matched-sibling HSCT is the preferred first therapy in children and young adults diagnosed with SAA, as transplant-related mortality and GVHD rates are lower in this younger age-group. GVHD and infection remain limiting factors to the success of transplantation, especially in older patients with severe AA. GVHD increases in frequency and severity in recipients >20 years of age, and more so >40 years of age. The increased risk of GVHD contributes to the poor survival in older patients, especially in those >40 years of age. The historically high rejection rate associated with AA patients appears to have improved to rates similar to those observed in patients transplanted for other conditions, likely because of less frequent use of immunogenic blood products and better conditioning regimens. Standard prophylactic therapy for GVHD includes a calcineurin inhibitor (cyclosporine or tacrolimus) and methotrexate.

In general, marrow transplantation using unrelated donors has resulted in higher morbidity and mortality than transplantations using matched sibling donors in AA and generally has been reserved for patients who lack a matched sibling donor and fail to respond to one or more rounds of ATG and cyclosporine therapy. Transplantation results with unrelated donors are typically best in younger patients (ie, children and young adults) who have not had significant infections or developed alloimmunization.

For older patients, reduced-intensity transplantation conditioning regimens using lower 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 children who fail an initial course of immunosuppression when a matched-unrelated donor is available.

Outcomes with mismatched-unrelated, haploidentical, or umbilical cord donors are not as favorable, with higher rates of graft rejection, infectious complications, acute and chronic GVHD, and transplant-related mortality. These higher risk transplants usually are undertaken when immunosuppression and other nonimmunosuppression strategies to improve blood counts have failed and no related- or unrelated-histocompatible donor is available.

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 increases.
- Bone marrow is the preferred source of stem cells in AA, not peripheral blood stem cells, unlike the situation with hematological neoplasms.
- Matched unrelated-donor transplantation should be reserved for patients for whom an initial course of immunosuppression has failed, especially in children and young adults

Immunosuppressive therapy

The principal immunosuppressive agent used in severe AA 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.

Initial investigations using horse ATG or cyclosporine alone in AA were succeeded by studies of horse ATG and cyclosporine in combination, with improved response rates with combination therapy. The addition of mycophenolate mofetil did not improve results, nor did the addition of sirolimus to standard horse ATG and cyclosporine.

The usual time to response to immunosuppressive therapy in SAA is approximately 10-12 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 horse ATG and cyclosporine is between 60% and 80%. Hepatitis-associated and drug-related AA appears to be equally responsive to immunosuppressive therapy as idiopathic AA. Although most patients who will respond to immunosuppressive therapy will 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 ATG have activity in SAA, with most of the experience with horse occurring in the upfront setting and experience with rabbit ATG in the salvage setting. A repeat cycle of rabbit ATG and cyclosporine may be given to refractory patients, which results in additional responses in approximately 35% of patients. In responders to horse ATG and cyclosporine, relapse has been reported in 35% of patients by 5 years. Relapses can be related temporally to the discontinuation of cyclosporine or to the reduction of the cyclosporine dose. Cyclosporine should be continued for at least 6 months. The benefit of a taper in reducing relapse rates has not been confirmed in prospective studies; however, most practicing hematologists institute a slow cyclosporine taper after 6 months in an attempt to prevent hematologic relapses. Relapsed patients may respond to an increased dose or reintroduction of cyclosporine or a second course of rabbit ATG, which results in hematologic responses in about 60%-70% of patients. Approximately 25% of patients remain chronically dependent on cyclosporine to maintain adequate blood counts.

The greater lymphocytotoxicity of rabbit 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 horse ATG. In a randomized study, however, results with rabbit ATG were disappointing. The hematologic response rate with rabbit ATG was 37% compared with 68% for horse ATG, and survival was inferior in the rabbit ATG arm. These results suggest that horse ATG plus cyclosporine should be preferred as first-line immunosuppressive therapy in severe AA.

As alternative therapy to ATG and cyclosporine, high-dose cyclophosphamide has been used, with response rates comparable to that of horse ATG but with apparently fewer rates of relapse and clonal evolution. This initial favorable experience with cyclophosphamide from a single center was not reproduced in a randomized study, however, where infectious complications in the cyclophosphamide arm were substantial, leading to prolonged severe neutropenia, hospitalizations, intensive care unit admissions, fungal infections, and deaths. In addition, relapses and clonal evolutions were observed in the cyclophosphamide arm in the randomized study.

More recently, alemtuzumab has shown activity in the salvage setting, producing a hematologic response in 30%-40% of those patients with AA refractory to initial horse ATG and about 50%-60% in patients with relapsed SAA. These results are similar to those reported with rabbit ATG in similar settings, which prompted a trial of alemtuzumab as initial therapy in SAA. The results, however, were disappointing because of a low response rate with alemtuzumab (~20%) compared with that of horse ATG plus cyclosporine (60%-70%).

Immunosuppression versus hematopoietic stem cell transplantation

Although an incomplete response, relapse, and clonal evolution to a clonal disease limit the success of ATG plus

cyclosporine, the morbidity and mortality of patients who receive HSCT can be substantial, primarily from infections and GVHD. Transplantation outcomes are age dependent. Currently, matched-sibling allogeneic marrow transplantation is the treatment of choice for children and adolescents, and may be considered as a first treatment option in adults up to the age of 40 years with severe AA, for whom transplantation-related morbidity and mortality are relatively low and potential remaining life span is relatively long (Figure 17-3). For patients without a matched-sibling donor or patients >40 years, immunosuppressive therapy is the initial treatment of choice.

In immunosuppression-refractory patients, sibling-donor HSCT may constitute a second-line therapy in selected older patients, and a matched-unrelated HSCT is a

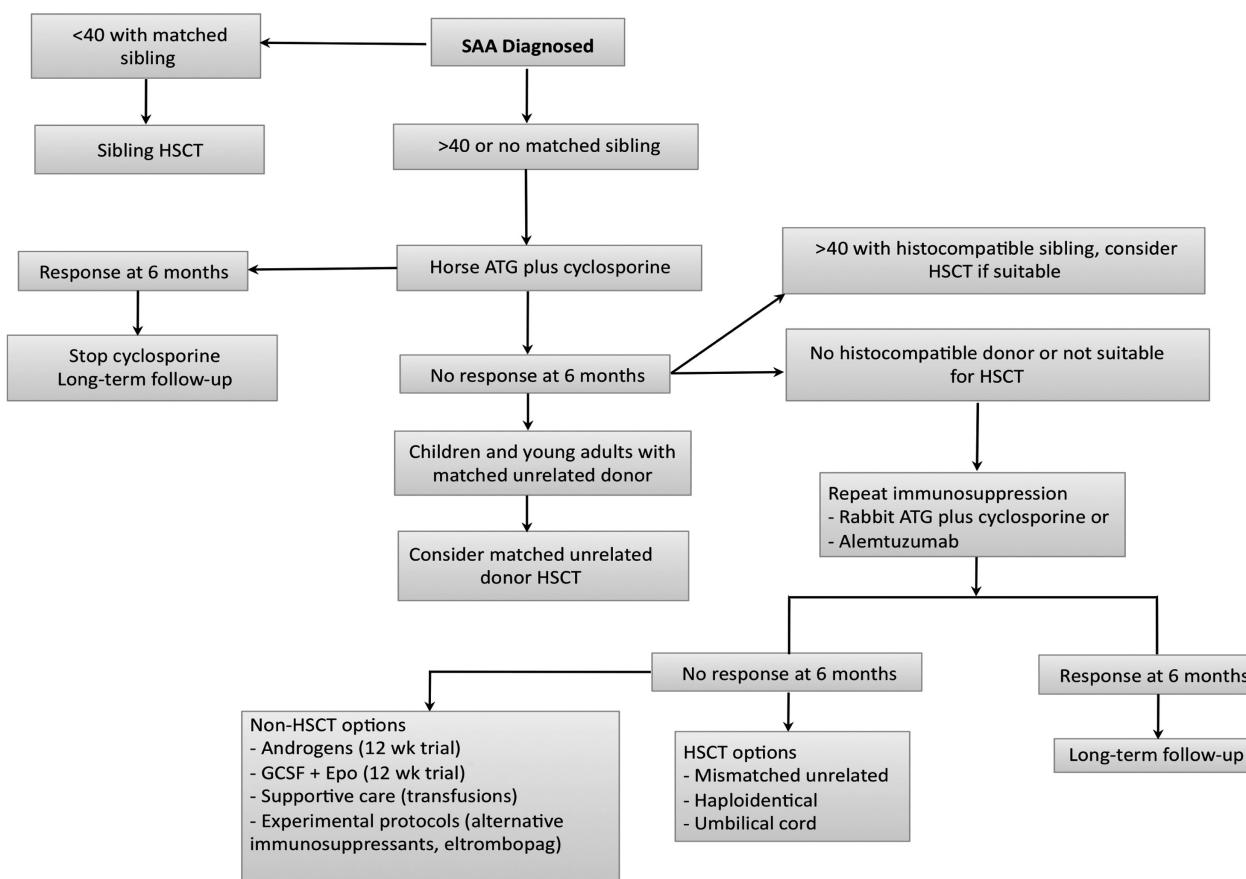


Figure 17-3 Algorithm for initial management of severe aplastic anemia (SAA). In patients who are not candidates for a matched related hematopoietic stem cell transplantation (HSCT), immunosuppression with horse antithymocyte globulin (ATG) plus cyclosporine should be the initial therapy. We assess for response at 3 and 6 months, but usually wait 6 months before deciding on further interventions in case of nonresponders. In patients who are doing poorly clinically with persistent neutrophil count $<0.2 \times 10^9/L$, we proceed to salvage therapies earlier between 3 and 6 months. Transplant options are reassessed at 6 months and donor availability, age, comorbidities, and neutrophil count become important considerations. We favor a matched unrelated HSCT in younger patients with a histocompatible donor, and repeat immunosuppression for all other patients. In patients with a persistently low neutrophil count in the very severe range, we may consider a matched unrelated donor HSCT in older patients. In patients who remain refractory after two cycles of immunosuppression, further management is then individualized taking into consideration suitability for a higher risk HSCT (mismatched unrelated, haploididential, or umbilical cord donor), age, comorbidities, neutrophil count, and overall clinical status. Some authorities in SAA consider 50 years of age as the cutoff for sibling HSCT as first-line therapy. Adapted from Scheinberg P, Young NS. How I treat acquired aplastic anemia. *Blood*. 2012;120(6):1185-1196.

viable option in younger patients. Despite significant progress with matched unrelated-donor marrow transplantation, data from large registries, however, suggest that survival rates remain inferior when compared with matched related-donor transplantation. Therefore, patients of all ages who do not have a matched-sibling donor should receive horse ATG plus cyclosporine as a first-line therapy.

Late clonal complications

Although a significant proportion of patients with AA will have PNH clones at presentation, the frequency of evolution of frank PNH has been reported to be as high as 20% in 10 years after initial diagnosis. 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%-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 immunosuppressive therapy.

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 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.

Key points

- Allogeneic stem cell transplantation from a matched sibling donor is the treatment of choice for patients with severe AA in children and young adults.
- For older patients, those without sibling donors, and those who refuse transplantation or have significant comorbidities that precludes HSCT, immunosuppression with horse 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, horse ATG plus cyclosporine should be the preferred initial treatment. Horse ATG is superior to rabbit 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.

Key points (continued)

- The combination of ATG and cyclosporine is more effective than single-agent immunosuppression in severe AA.
- Relapses occur in about one-third of responders to horse ATG plus cyclosporine but often respond well to reinstitution of immunosuppressive therapy.
- Repeat courses of rabbit ATG and cyclosporine may be given to refractory patients, resulting in a salvage rate of approximately 35%.
- Clonal evolution to MDS can occur in 10%-20% of patients long term.
- Higher risk transplant modalities from mismatched-unrelated, haploididentical, or umbilical cord donors should be reserved for patients refractory to two or more courses of immunosuppression who remain severely pancytopenic.

Paroxysmal nocturnal hemoglobinuria

Definition

PNH is a clonal bone marrow failure disorder resulting from a somatic mutation in the *PIGA* gene in HSCs, which results in failure to synthesize the glycophosphatidylinositol (GPI) anchor. The consequence of failure to synthesize this anchor is a deficiency of all GPI-anchored proteins on the surface of progeny cells of all hematopoietic lineages derived from the affected stem cell (Figure 17-4). Clinically, PNH is characterized by a triad of signs: intravascular hemolysis, hypercoagulability, and bone marrow failure.

Pathophysiology

There are two classes of cell membrane-associated proteins: transmembrane proteins and GPI-anchored proteins. In PNH, because of the defect of the enzyme encoded by the mutant *PIGA* gene, the first step in biosynthesis of the GPI anchor cannot be completed, and all GPI-anchored proteins are absent in the membrane of affected cells. Abnormalities in the *PIGA* gene identified in patients with PNH include deletions, insertions, and missense, nonsense, and splice-site mutations. *PIGA* mutations have been found in asymptomatic individuals, and patients with PNH may harbor multiple clones with distinct *PIGA* mutations.

The relative size of the PNH clone as measured by flow cytometry correlates generally with the severity of disease; clinically apparent PNH is common in patients with >50% GPI-deficient cells, whereas those with <10% GPI-deficient cells usually have "subclinical" PNH. Although cells have many GPI-anchored proteins with diverse functions, it is hypothesized that symptoms of PNH are related to the deficiency of specific GPI-linked proteins, such as those that protect cells from complement-mediated lysis (Figure 17-5).

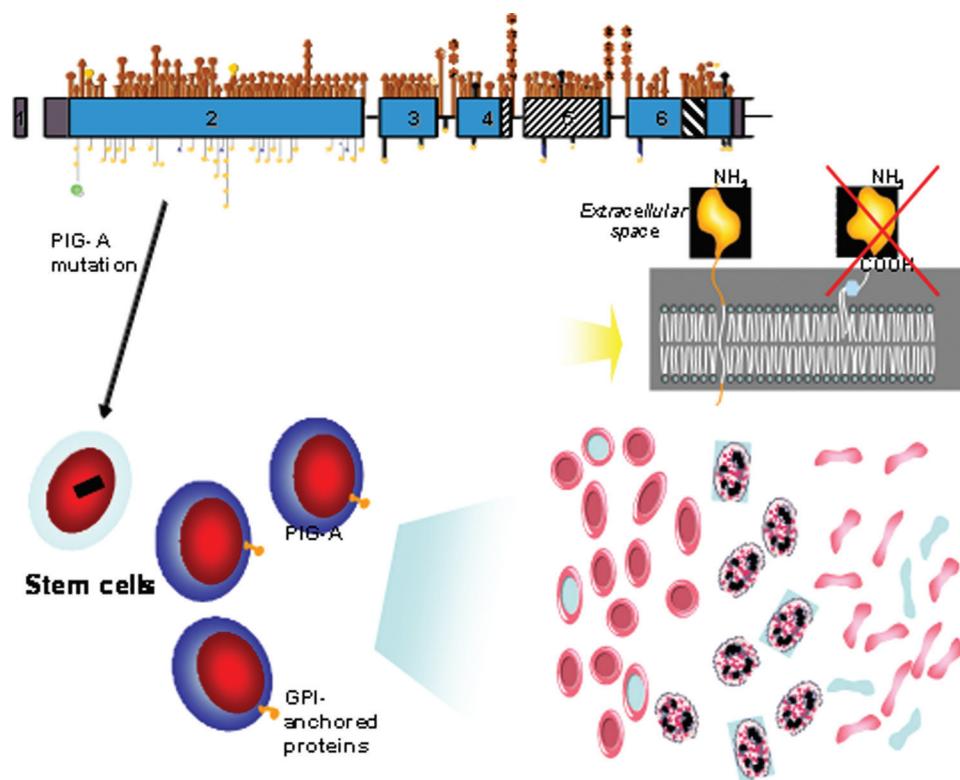


Figure 17-4 Pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH). In hematopoietic stem cells, acquired somatic mutations of the *PIGA* gene may occur. Mutations occur across the gene and without a specific hot spot. Such mutations can decrease the function or totally inactivate the enzyme encoded by *PIGA*, which controls the key step in the biosynthesis of glycosphingomylin (GPI) in the lip anchor of the GPI-linked class of membrane proteins. As a consequence, all proteins using this type of anchor are deficient from the membrane of affected progeny derived from the mutant stem cells. With the expansion of PNH clone, presumably because the clone can evade an aberrant immune response, the contribution of normal stem cells to blood cell production decreases.

Hemolysis

Intravascular hemolysis in PNH is due to the lack of a decay-accelerating factor (DAF; CD55) and membrane inhibitor of reactive lysis (MIRL; CD59), proteins 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 between patients and in an individual patient over time. The enhanced hemolysis observed during infections can be accounted for by increased complement activation on the red blood cell surface.

Thrombosis

Several theories have been postulated to account for the hypercoagulability observed in PNH patients, but the mechanism

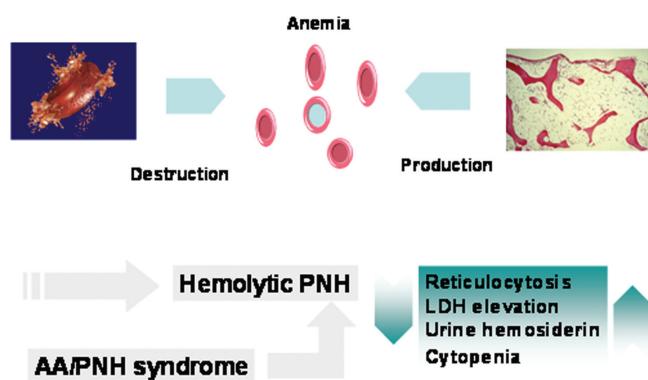


Figure 17-5 Pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH)-associated anemia. Anemia in PNH can be a result of increased red blood cell (RBC) destruction due to intravascular hemolysis of glycosphingomylin (GPI)-deficient RBCs, decreased production of RBCs due to immune-mediated bone marrow failure, or a combination of these 2 mechanisms. Hemolysis can be compensated for by increased production (patients with increased reticulocytes), or compensation may be inadequate (patients with low reticulocyte counts). Hemolytic PNH can develop from aplastic anemia (AA) or myelodysplastic syndrome (eg, AA/PNH syndrome), or it can be a primary disease.
LDH = lactate dehydrogenase

has not been clearly defined. It is believed that thrombophilia in PNH is related to the degree of hemolysis and thereby indirectly to the size of PNH clone. Possible pro-thrombotic pathways include platelet activation by complement components, procoagulable microparticles derived from GPI-deficient erythrocytes, lack of GPI-anchored urokinase plasminogen activator receptor, slowing of the microcirculation because of vasoconstriction induced by products of hemolysis, and deficiency of proteinase 3 that normally is displayed on neutrophils via GPI-anchored CD177 (proteinase 3 decreases the exposure of an epitope on platelet protease activated receptor-1 that is needed for thrombin activation, so lack of proteinase 3 indirectly increases thrombin activation). It also has been suggested that intravascular hemolysis exposes red blood cell phospholipids that may serve to initiate coagulation.

Bone marrow failure and evolution of PNH

PNH clones evolve only in the context of immune-mediated bone marrow failure, 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 enjoy selective survival advantage compared with healthy stem cells, which facilitates their expansion. The molecular nature of this growth advantage has not been clarified, but it likely is related to a deficiency of certain immunomodulatory GPI-anchored proteins from the surface of PNH stem cells. 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.

Laboratory findings and diagnosis

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.

The laboratory diagnosis of PNH formerly relied on the demonstration of abnormally complement-sensitive eryth-

rocyte populations. Thomas Hale Ham first described the acidified serum lysis test in 1938. In that test, acidification of the serum activates the alternative pathway of complement, and increased amounts of C3 are fixed to red blood cells lacking complement regulatory proteins. Complement sensitivity of PNH red blood cells also can be demonstrated in high-concentration sucrose solutions, which is the basis for the “sugar water” or sucrose lysis test. These two tests are primarily of historical interest because they lack sensitivity; even in the presence of significant hemolysis, PNH clones can be missed with the Ham and sucrose lysis tests.

Currently, the diagnosis of PNH is secured by flow cytometry, in which the percentage of circulating granulocytes deficient in GPI-anchored proteins is assayed. PNH granulocytes are defined by the absence of two otherwise-constitutive GPI-anchored proteins, such as CD55, CD59, CD66b, or CD16. Flow cytometric methods are sensitive and can detect even very small PNH clones (<1%) that are of no clinical significance. Because hemolysis can underestimate the proportion of GPI erythrocytes, GPI granulocytes are used as a better determinant of the PNH clone size. An exceptionally sensitive flow cytometric assay that makes use of fluorescent-labeled aerolysin (FLAER, a toxin with high affinity for the GPI anchor) has been developed, but the clinical relevance of identifying tiny clones (not detected by conventional flow cytometry) is still uncertain.

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 may experience chronic hemolytic anemia, cytopenias, and a thrombotic tendency.
- Chronic hemolysis-associated urinary hemosiderin losses may result in iron deficiency.
- Flow cytometric techniques to identify cell populations lacking GPI-linked proteins, such as CD55 and CD59, have replaced the obsolete Ham and sucrose lysis tests for 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; this symptom correlates with the size of the PNH clone. 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. 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. Reported fatigue is worse than expected from the degree of anemia and has been hypothesized to be related to impaired microcirculation resulting from microthrombi or vasoconstriction associated with hemolysis. In the presence of coexistent bone marrow failure, reticulocytosis may be absent, and patients may display various degrees of pancytopenia. In some patients, however, anemia may be mild and well compensated.

The most concerning complication of PNH is thrombosis, which can occur at unusual sites, including mesenteric, hepatic, splenic veins, or cerebral (dural) sinus thrombosis. PNH can be associated with the Budd-Chiari syndrome, and a diagnosis of PNH often is missed in this setting. For unclear reasons, thrombotic complications are less common in PNH patients of Asian descent. The thrombotic propensity is particularly enhanced during pregnancy. Conceptually, PNH can be classified as follows:

- Primary hemolytic PNH
- Secondary hemolytic PNH (history of antecedent AA)
- AA/PNH syndrome (ie, coexistence of a sizable PNH clone in a patient with bone marrow failure and a hypocellular marrow)
- Small subclinical PNH clones detected in the context of otherwise typical AA or MDS, which are of uncertain significance but should be monitored because they can expand and come to dominate the clinical picture

Treatment

Except for allogeneic HSCT, there are no curative treatments for PNH, but long-term remissions are possible. 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 bone marrow failure-related anemia. Chronic hemolysis should be treated with supportive measures, such as supplementation of folate and iron, and, in the context of renal failure, recombinant erythropoietin administration. Some patients may benefit from low-dose or alternating doses of prednisone or androgenic steroids. Acute hemolytic attacks may require hydration, increased doses of corticosteroids, and transfusions.

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. Treatment with eculizumab is associated with few complications, but because

the terminal components of complement are important to protect from *Neisseria meningitis*, vaccination against this organism is important before initiation of eculizumab therapy. 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.

The clinician should have a high index of suspicion for thrombosis in patients with PNH who develop new symptoms potentially consistent with a clotting event. Conversely, patients who present with thrombotic complications in unusual sites should be investigated for the presence of PNH, especially if anemia is present. Thrombosis is a major source of morbidity and mortality in patients with PNH.

Once the diagnosis of a thrombosis is made, aggressive treatment is warranted. Thrombolytic therapy is effective in the management of hepatic vein thrombosis and also should be considered as an initial option for other major venous thrombotic events. The administration of heparin followed by chronic warfarin using standard approaches is recommended. Unless the patient undergoes therapy resulting in elimination of the PNH clone (as discussed later in this section), indefinite anticoagulant therapy should be considered for patients who have had a thrombotic event.

For the patient without thrombosis, prophylactic therapy is indicated for provocative clinical settings (eg, surgery and prolonged immobilization), as for other hypercoagulable conditions. A prospective study has suggested that patients with a large PNH clone (ie, PNH granulocytes >50%) and no contraindication to anticoagulation benefit with a substantial reduction of spontaneous thrombosis when given prophylactic warfarin. Preventive anticoagulation of all PNH patients remains controversial, however; thrombotic complications occur in only ~30% of patients over a lifetime. In some patients, anticoagulation may not be possible because of the presence of thrombocytopenia.

Recently, in a nonrandomized study, eculizumab appeared to decrease the rate of thrombosis in a cohort of PNH patients in whom clotting events were compared before and after the use of eculizumab. This suggests a close pathophysiologic link between hemolysis and thrombosis. It remains unclear, however, whether patients receiving eculizumab may discontinue chronic anticoagulation that had been instituted for a thrombotic event.

The approach to bone marrow failure associated with PNH should be similar to that taken for severe AA. Immunosuppressive therapy with horse 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.

Allogeneic HSCT appears to be the only curative therapy modality for PNH. Although only small series of PNH patients who have received transplantation have been published, the outcomes appear to be less favorable than in AA. Application of reduced-intensity transplantation approaches may change this situation. More widely accepted indications for HSCT in PNH include the presence of severe bone marrow failure and intractable thrombotic events despite adequate therapy, a scenario increasingly infrequent with the activity of eculizumab as antithrombotic in PNH.

Prognosis

The median survival time for patients with PNH is 10–15 years. Thrombotic events, progression to pancytopenia, and age >55 years at diagnosis are poor prognostic factors. The development of an MDS or acute leukemia markedly shortens survival. Patients without leukopenia, thrombocytopenia, or other complications can anticipate long-term survival.

Key points

- Steroid therapy is controversial but may ameliorate bouts of hemolytic anemia in some cases.
- 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.
- Prompt evaluation of PNH patients is indicated when symptoms are suggestive of thrombosis because the risk of clotting is high.
- PNH can be a cryptic cause of unusual thrombotic events such as splenic vein thrombosis, cerebral sinus thrombosis, or Budd-Chiari syndrome.
- Thrombotic complications of PNH require indefinite anticoagulation, which has been shown to decrease the rate of recurrent thrombosis.
- Treatment of bone marrow aplasia with immunosuppressive therapy 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.

Myelodysplastic syndromes

Clinical case

A 78-year-old retired firefighter with an unremarkable past medical history develops fatigue and exertional dyspnea. Physical examination demonstrates generalized pallor; splenomegaly and lymphadenopathy are absent. A complete blood count reveals a hemoglobin level of 8.1 g/dL, white blood

Clinical case (continued)

cell count of $2.9 \times 10^9/L$ with 33% neutrophils, and a platelet count of $88 \times 10^9/L$. Serum vitamin B12 and red blood cell folate levels are normal; the ferritin level is 348 ng/mL. Peripheral smear shows hypogranular, hypolobated neutrophils. The patient undergoes marrow aspiration and biopsy, which reveals a hypercellular marrow for age (80% cellularity) with erythroid hyperplasia, megaloblastoid erythroid maturation, reduced granulocyte progenitors, and scattered abnormal hypolobated megakaryocytes. There are 8% blasts in the bone marrow that express myeloid markers, and the karyotype is 47, XY, +8 [12], 46, XY, del(20)(q11q13) [8].

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 as ≥20% blast cells in the marrow or blood; thus, all patients with MDS have <20% marrow blasts, by definition.

MDS may arise de novo (~85% of cases are idiopathic) or may be secondary to a recognized exposure to DNA-damaging agents. Secondary or therapy-related MDS (t-MDS) can be due to drugs that alkylate DNA bases (eg, chlorambucil, cyclophosphamide, melphalan), to therapeutic ionizing radiation, to inhibitors of topoisomerase II (eg, topotecan, etoposide, anthracyclines), or to environmental or occupational exposure to other DNA toxins, such as kerosene or benzene. Proving a connection between a suspect exposure and subsequent development of MDS can be difficult, but the presence of a complex karyotype (≥3 acquired chromosome abnormalities) or abnormalities of chromosomes 5 and 7 is suggestive of secondary MDS.

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 (Figure 17-6; discussed further later) 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 large numbers of these “dud” cells are present. As a result, the infection and bleeding risks in MDS correlate only generally with the circulating neutrophil and

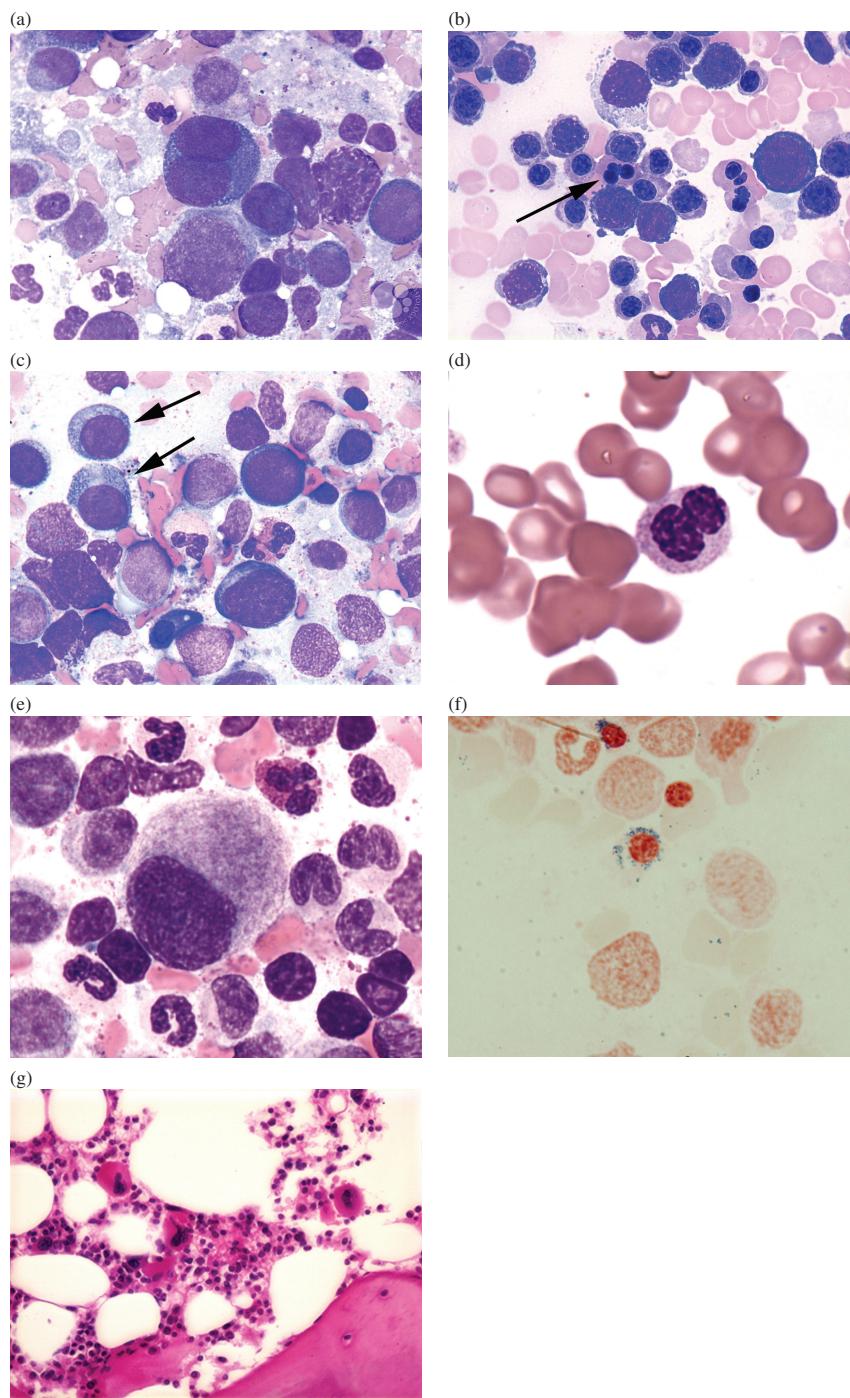


Figure 17-6 Typical blood and marrow cell morphology in patients with MDS.

(a) and (b) Multinuclear red blood cell precursors (*arrows*) at different stages of maturation. These cells are characteristic of MDS. (a) Source: ASH Image Bank #00001151. (b) Source: ASH Image Bank (2006); doi: 10.1182/ashimagebank-2006-6-00037, Figure 7. (c) Asynchronous maturation of erythroid cells (*arrows*). The chromatin pattern of these cells is fine, suggesting relative immaturity, whereas the lightening of the cytoplasm indicative of hemoglobinization is an event associated with later stages of maturation. Source: ASH Image Bank (2006); doi: 10.1182/ashimagebank-2006-6-00038, Figure 6. (d) Hypolobated neutrophil (pseudo-Pelger-Huët cell) found in the peripheral blood of a patient with refractory anemia. Source: ASH Image Bank (2004); doi: 10.1182/ashimagebank-2004-101151. (e) Micromegakaryocytes may have an eccentric, hypolobulated or round nucleus. Source: ASH Image Bank (2004); doi: 10.1182/ashimagebank-2004-101142. (f) Ring sideroblast (a Prussian blue reaction on a marrow aspirate, seen at low power magnification). Source: ASH Image Bank (2006); doi: 10.1182/ashimagebank-2006-6-00022. (g) The bone marrow biopsy in this middle-aged woman with isolated del(5q) reveals a marrow that is normocellular with increased numbers of megakaryocytes that have hypolobulated nuclei (hematoxylin and eosin stain). Source: ASH Image Bank (2004); doi: 10.1182/ashimagebank-2004-101163.

platelet count, and some MDS patients with severe cytopenias are less symptomatic than other patients who have more modest cytopenias. The bone marrow in MDS usually is normocellular or hypercellular for age, but 10%–20% of cases are accompanied by a hypocellular marrow, and such cases of hypoplastic MDS may be difficult to distinguish from AA.

Classification

The current prevailing classification of MDS is the fourth edition of the World Health Organization (WHO) *Classification of Tumors of Hematopoietic and Lymphoid Tissues*, published in 2008 (Table 17-4). The 2008 WHO MDS classification is a minor modification of the third edition WHO classification,

Table 17-4 2008 World Health Organization (WHO) classification of myelodysplastic syndromes and neoplasms.

Name	Abbreviation	Peripheral blood: key features	Bone marrow: key features	WHO-estimated patients with MDS (%)
Refractory cytopenias with unilineage dysplasia (RCUD):				
<i>Refractory anemia</i>	RA	Anemia; <1% peripheral blood blasts	Unilineage erythroid dysplasia (in >10% of cells); <5% blasts	10%-20%
<i>Refractory neutropenia</i>	RN	Neutropenia; <1% peripheral blood blasts	Unilineage granulocytic dysplasia; <5% blasts	<1%
<i>Refractory thrombocytopenia</i>	RT	Thrombocytopenia; <1% blasts	Unilineage megakaryocytic dysplasia; <5% blasts	<1%
Refractory anemia with ring sideroblasts	RARS	Anemia; no blasts	Unilineage erythroid dysplasia; ≥15% of erythroid precursors are ring sideroblasts; 5% blasts	3%-11%
Refractory cytopenias with multilineage dysplasia	RCMD	Cytopenia(s); <1% blasts; no Auer rods	Multilineage dysplasia ± ring sideroblasts; <5% blasts; no Auer rods	30%
Refractory anemia with excess blasts, type 1	RAEB-1	Cytopenia(s); <5% blasts; no Auer rods	Unilineage or multilineage dysplasia; 5%-9% blasts; no Auer rods	40%*
Refractory anemia with excess blasts, type 2	RAEB-2	Cytopenia(s); 5%-19% blasts; ± Auer rods	Unilineage or multilineage dysplasia; 10%-19% blasts; ±Auer rods	
Myelodysplastic syndrome (MDS) associated with isolated del(5q)	Del(5q)	Anemia; normal or high platelet count; <1% blasts	Isolated 5q31 chromosome deletion; anemia, hypolobated megakaryocytes	<5%
Childhood MDS, including refractory cytopenia of childhood (provisional)	RCC	Pancytopenia	<5% marrow blasts for RCC; marrow usually hypocellular	<1%
MDS, unclassifiable	MDS-U	Cytopenias; ≤1% blasts	Does not fit other categories; dysplasia; <5% blasts; if no dysplasia, MDS-associated karyotype	?

Note: If peripheral blood blasts are 2%-4%, the diagnosis is RAEB-1 even if marrow blasts are <5%. If Auer rods are present, the WHO considers the diagnosis RAEB-2 if the blast proportion is <20% (even if <10%) or acute myeloid leukemia (AML) if ≥20% blasts. Cases of RCUD, RARS, or RCMD where the peripheral blood blasts are exactly 1% should be considered MDS-U, according to the WHO. For all subtypes, peripheral blood monocytes are <1 × 10⁹/L. Bicytopenia may be observed in RCUD subtypes, but pancytopenia with unilineage marrow dysplasia should be classified as MDS-U. Therapy-related MDS (t-MDS), whether due to alkylating agents, topoisomerase II inhibitors, or radiation, is classified together with therapy-related AML (t-AML) in the WHO classification of AML and precursor lesions. The listing in this table excludes MDS/myeloproliferative neoplasm overlap categories, such as chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, and the provisional entity RARS with thrombocytosis.

*This 40% figure represents the proportion of patients with RAEB-1 or RAEB-2, collectively.

From Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:87-107.

formally published in 2001, which in turn was built on the 1982 MDS classification by the French-American-British (FAB) Cooperative Group. Important classification factors in the current WHO MDS schema include the specific myeloid cell lineages in which >10% of cells are dysplastic, the marrow and peripheral blood blast proportion, whether or not ≥15% of erythroid precursor cells in the marrow are ring sideroblasts (an arbitrary threshold), and, to a limited extent, the presence of cytogenetic abnormalities, such as interstitial deletion of the long arm of chromosome 5. By definition, MDS are associated with <20% marrow and blood blasts. The WHO has group t-MDS with therapy-related AML (t-AML) because the outcome in such patients is poor regardless of the blast count.

The observation that alkylating agents, topoisomerase inhibitors, and ionizing radiation predispose patients to both MDS and AML and the existence of shared cytogenetic abnormalities, such as deletions or gains in all or parts of chromosomes 5, 7, 8, or 20, 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. The so-called good-risk recurrent AML-associated translocations, t(8;21), t(15;17), and inv(16), are extremely 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%-30% likelihood overall, with some subtypes of MDS at greater risk), but most patients with MDS do not develop AML. Instead, most 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, some patients succumb to unrelated conditions that are common in the elderly, and 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 of HSCs across the human life span. Eventually a mutation or combination of mutations occur in such a way in a given hematopoietic cell that its progeny acquire a growth and survival advantage, and that expanded clone of cells is then at risk for acquiring additional mutations that increase its malignant potential. The median age at diagnosis is ~70 years. Overall, there is a slight male predominance in MDS that possibly is related to occupational exposures, but a

specific MDS subtype, MDS associated with isolated deletion of the long arm of chromosome 5 and a specific marrow morphology, including hypolobated megakaryocytes (*5q-syndrome*), is more common in women than in men.

MDS are rare in the pediatric age-group and represent ~5% of hematologic malignancies in patients <18 years of age. When MDS do arise in children, they frequently are associated with Down syndrome, with congenital marrow failure syndromes, or with germline defects of DNA repair, such as Li-Fraumeni syndrome or Bloom syndrome. Children with Shwachman-Diamond syndrome, congenital neutropenia, or FA are at markedly increased risk of developing MDS. In all of these inherited conditions, MDS arise in the context of hematopoietic deficits and typically present 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-RCC).

Refractory anemia with excess blasts is also relatively common 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. Refractory anemia with ring sideroblasts (RARS) 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 because of germline mutations of *ALAS2*, which does not carry a risk of progression to AML. Familial MDS or AML with monosomy 7 has been reported in at least 10 families in the absence of either phenotypic abnormalities or any history of hematologic disorders. Germline mutations in *RUNX1* and *GATA2* transcription factors also predispose to MDS. Germline *RUNX1* mutations are associated with a prodrome of thrombocytopenia. *GATA2* mutations are often nonsyndromic but can be associated with mycobacterial infections, lymphedema, and monocytopenia (MonoMAC syndrome).

In most adult patients with MDS, the etiology is unknown, and there is no specific predisposing factor identifiable other than advanced age. Occupational exposure to organic solvents, such as benzene, is associated with the development of MDS, but such exposures rarely occur in industrial nations.

Key points

- MDS are characterized by ineffective hematopoiesis, leading to peripheral blood cytopenias. The marrow is often hypercellular for age.
- Aging and exposure to alkylating agents, topoisomerase II inhibitors, or ionizing radiation are all risk factors for developing MDS.
- MDS are rare in children, and when they occur, are often associated with congenital marrow failure syndromes.

Key points (continued)

- Germline mutations in *RUNX1* and *GATA2* are associated with a subsequent risk for MDS development.
- The 2008 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 peripheral 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. The diagnosis of MDS is based primarily on morphologic criteria demonstrating dysplastic features in the peripheral blood and $\geq 10\%$ of bone marrow precursor cells in one or more lineages—erythroid, myeloid, megakaryocytic (Figure 17-6).

In one large study, the median hemoglobin of patients diagnosed with MDS was 9.5 g/dL, and 75% of patients had a level < 11 g/dL. Only 20% of patients had both a platelet count $> 100 \times 10^9/\text{L}$ and an absolute neutrophil count $> 1.0 \times 10^9/\text{L}$, 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 are asymptomatic at diagnosis and are discovered only when a complete blood count is performed 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 bone 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 17-6). 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. 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 specific aberrations correlate with prognosis and response to treatment (see Table 17-5). In a small percentage of cases, fluorescence *in situ* hybridization (FISH) analysis reveals specific chromosomal translocations and losses or gains of DNA segments that were not detected with standard cytogenetic methods. The clinical relevance of small clones

Table 17-5 The 1997 International Prognostic Scoring System (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	5-10	—	11-20	21-30*
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	—	—	—

*No longer considered myelodysplastic syndrome (redefined as acute myeloid leukemia by World Health Organization in 2001).

†IPSS definition of peripheral blood cytopenias: hemoglobin < 10 g/dL; absolute neutrophil count $< 1800/\mu\text{L}$; and platelet count $< 100000/\mu\text{L}$.

Scoring system: A point value from 0 to 2.0 is determined for each of the three prognostic factors in Table 17-6, and the three values are summed to obtain the total IPSS score.

From Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*. 1997;89:2079-2088.

detectable only by FISH is uncertain. The yield of FISH is very low if karyotyping is successful.

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. 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. 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 differential from the marrow aspirate.

In the near future, array-based testing and broad-based molecular profiling will become increasingly important in MDS diagnosis, especially in ambiguous cases, and in prognostic assessment. Detection of an SF3B1 mutation, for instance, would support a diagnosis of RARS rather than a congenital sideroblastic anemia or a reactive cause of sideroblastic anemia.

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.

Key points

- Complete blood counts, peripheral blood morphology, bone marrow aspirate and core biopsy, and cytogenetic testing are key to establishing 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.
- 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.
- Anemia is the most common cytopenia in patients with MDS.
- Functional defects of neutrophils and platelets exacerbate the clinical problems associated with neutropenia and thrombocytopenia.

Idiopathic cytopenias of undetermined significance

Some patients present with cytopenias and nondiagnostic bone marrow findings (ie, minimal or no dysplastic changes and no increase in marrow myeloblasts) and without any

cytogenetic abnormalities—a situation in which MDS is possible and an alternative diagnosis is not apparent, yet WHO-defined diagnostic criteria for MDS are not met. Such patients have been termed as having *idiopathic cytopenia(s) of undetermined significance* (ICUS). Close observation of patients with ICUS is recommended. Some patients with ICUS will develop overt MDS or AML over time, whereas others eventually will be found to have an alternative diagnosis; many will be stable for a prolonged period, with or without complications from the cytopenias. Unlike other undetermined-significance hematologic conditions (eg, monoclonal gammopathy of undetermined significance, monoclonal B-cell lymphocytosis of undetermined significance), patients with ICUS are, by definition, not known to have a clonal disorder.

A related situation occurs when patients have cytopenias and a nondiagnostic bone marrow, yet a clonal cytogenetic abnormality typical for MDS (eg, del[5q]) is present. The clinical behavior of such patients is typical for MDS, with a risk of death from cytopenias and progression to AML. In the 2008 version of the WHO classification of MDS, such patients with unremarkable morphology but an abnormal karyotype were considered as having MDS, unclassifiable subtype. Certain karyotypes (eg, trisomy 8, del[20q], and loss of the Y chromosome) are not specific enough to define a case as MDS in the absence of dysplastic morphology.

Prognosis

In 1997, the International Prognostic Scoring System (IPSS) (Tables 17-5 and 17-6) 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.

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 $9 \times 10^9/L$ is not weighted any differently by the IPSS than a count of $90 \times 10^9/L$, although several studies have shown that severe thrombocytopenia is an important risk factor for disease progression and death. The IPSS has been validated 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 2008

Table 17-6 Risk stratification of International Prognostic Scoring System (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, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*. 1997;89:2079-2088.

WHO classification system for individual patients, and it remains widely used for clinical trial enrollment purposes.

Several newer MDS prognostic systems have been introduced since 2007 to try to overcome limitations of the IPSS. These newer risk stratification models include the WHO-based Prognostic Scoring System (WPSS), which integrates the WHO classification with karyotyping data and the degree of anemia; a modified form of the WPSS 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 >7000 patients from more than 10 countries (Table 17-7, Table 17-8, and Figure 17-7). The primary 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 only 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 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.

Key points (continued)

- 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

Chromosome analysis provides strong evidence that MDS are clonal disorders. Approximately one-half of patients with de novo MDS and most patients with secondary MDS have cytogenetic abnormalities detectable on routine G-banded karyotyping. Cytogenetic results have independent prognostic significance (Table 17-7). 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). Such techniques have highlighted the diversity of MDS and may help better define the molecular biology of MDS in the years to come.

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 in recent years because patients with deletions of chromosome 5q preferentially respond to lenalidomide therapy (see section on treatment). Haploinsufficiency of a 5q-encoded ribosomal protein, RPS14, contributes to

Key points

- The IPSS is 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.

Table 17-7 MDS cytogenetic risk stratification system used in the revised International Prognostic Scoring System (IPSS-R).

Updated cytogenetic classification for use in IPSS-R (n = 7012)					
Risk group	Included karyotypes (19 categories)	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, 2 or more independent clones	2.7	2.5	13%	
Poor	Abnormal 3q, -7, double abnormality include -7/del(7q), complex with 3 abnormalities	1.5	1.7	4%	
Very poor	Complex with >3 abnormalities	0.7	0.7	7%	

AML = acute myeloid leukemia; IPSS-R = International Prognostic Scoring System–Revised; N/R = not reached.

Greenberg P, et al. *Blood*;120:2454–2465. [Epub 27 June 2012.]

Parameter	IPSS-R				
	Categories and Associated Scores				
Cytogenetic risk group	Very good	Good	Intermediate	Poor	Very Poor
	0	1	2	3	4
Marrow blast proportion	≤2%	2–<5%	5–10%	>10%	
	0	1	2	3	
Hemoglobin	≥10 g/dL	8–<10 g/dL	<8 g/dL		
	0	1	1.5		
Absolute neutrophil count	≥0.8 × 10 ⁹ /L	<0.8 × 10 ⁹ /L			
	0	0.5			
Platelet count	≥100 × 10 ⁹ /L	50–100 × 10 ⁹ /L	<50 × 10 ⁹ /L		
	0	0.5	1		

Possible range of summed scores: 0–10

IPSS-R = International Prognostic Scoring System–Revised.

Greenberg P, et al. *Blood*;120:2454–2465. [Epub 27 June 2012.]

Table 17-8 2012 Revised International Prognostic Scoring System for MDS (IPSS-R).

defective erythropoiesis, just as germline mutations of ribosomal components contribute to DBA (see section on DBA in Chapter 15, “Myeloid disorders and congenital marrow failure syndromes”). As originally described, the 5q– syndrome is associated with erythropoietin-refractory macrocytic anemia, normal or increased platelet count, giant platelets, dyserythropoiesis, 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. Patients with the 5q– abnormality 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 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 myeloproliferative neoplasms (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, 13q, 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 more than recurrent point

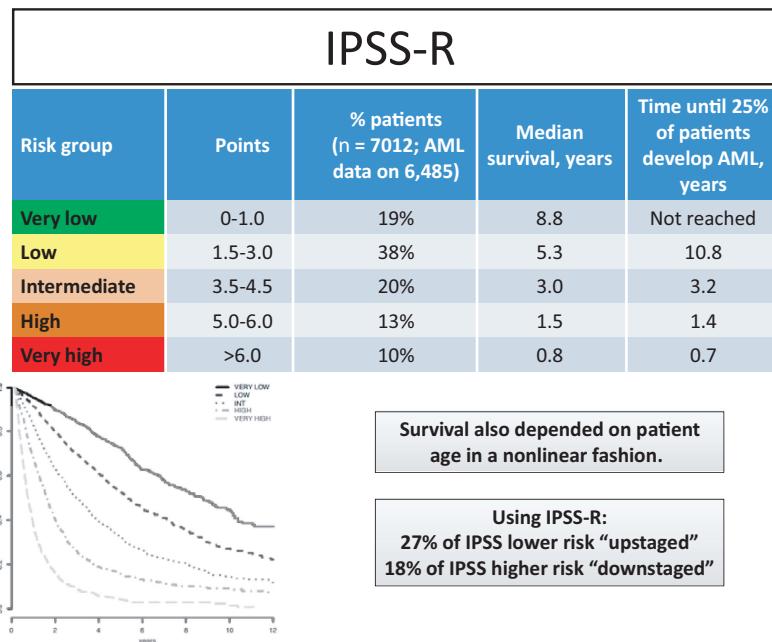


Figure 17-7 Tabular and graphical representation (Kaplan-Meier survival analysis) of the five risk groups defined by the IPSS-R. AML = acute myeloid leukemia; IPSS-R = International Prognostic Scoring System-Revised. Greenberg P, et al. *Blood*; 120:2454-2465. [Epub 27 June 2012.]

mutations in more than 20 different genes, some of which are shared with AML and other neoplasms (Figure 17-8). These techniques also demonstrate that the majority of cells in the marrow are clonal even in lower-risk MDS.

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* 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 in 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%-10% of MDS cases, especially t-MDS. *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%-25% 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 RARS. Other common splicing mutations include *SRSF2* and *U2AF1*.

More than 70% of patients with MDS have at least one somatic mutation detectable in hematopoietic cells. Several of these mutations have IPSS-independent prognostic

significance. For instance, patients with mutations in *TP53*, *NRAS*, *RUNX1*, *ASXL1*, or *EZH2* have an increased risk of leukemia progression or death. Patients with IPSS low-risk disease who harbor one of these mutations have an outcome more similar to IPSS intermediate-1-risk disease.

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 3-7 years. Patients treated with epipodophylotoxins (eg, etoposide) can develop specific translocations involving the breakpoint at 11q23; the latency period between exposure and MDS/AML development is typically 1-3 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 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,

MDS mutation landscape

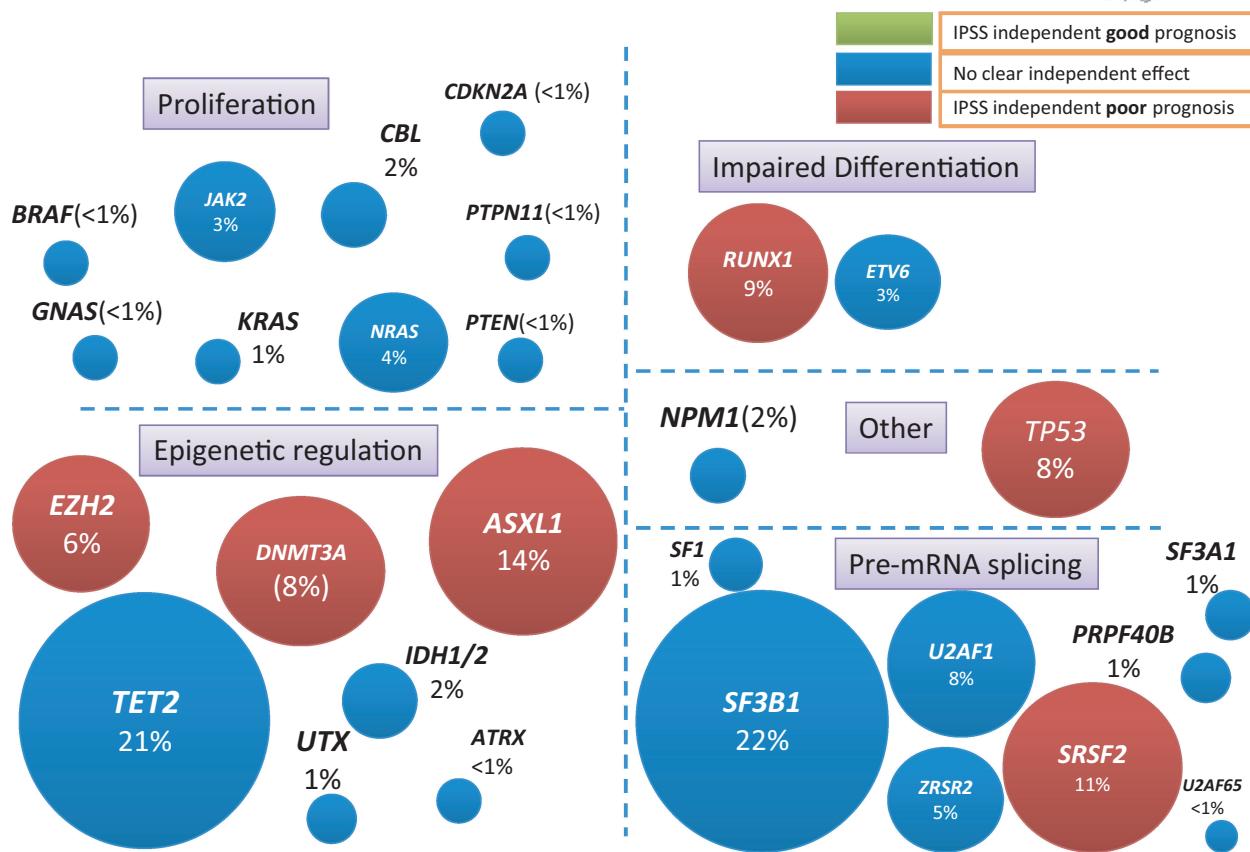


Figure 17-8 Recurrent somatic mutations in MDS. Approximate frequency of 27 recurrent somatic mutations in MDS and their prognostic significance. Some mutations influence the phenotype and are therefore more common in specific subtypes of MDS; for instance, *SF3B1* mutations are found in >50% of patients with RARS, *ATRX* mutations are most common in MDS with acquired alpha thalassemia, and *SRSF2* mutations are more common in MPN/MDS overlap syndromes such as chronic myelomonocytic leukemia. IPSS = International Prognostic Scoring System; MDS = myelodysplastic syndromes.

Frequency of *TET2*, *EZH2*, *ASXL1*, *IDH2*, *NRAS*, *KRAS*, *JAK2*, *BRAF*, *GNAS*, *CBL*, *CDKN2A*, *PTPN11*, *RUNX1*, *ETV6*, *NPM1*, *PTEN*, and *TP53* mutations and their prognostic significance are derived from Bejar R, Stevenson K, et al. *N Engl J Med.* 2011; Jun 30;364(26):2496-2506. Frequency of *SF3B1*, *ZRSR2*, *SRSF2*, *SF3A1*, *PRPF40B*, *USA65*, *U2AF1* (=*U2AF35*), and *SF1* mutations are from Yoshida K, Sanada M, et al. *Nature* 2011 October 6;478:64-69 (doi:10.1038/nature10496), with *SF3B1*, *ZRSR2*, *SRSF2*, and *U2AF1* data frequency combined with Thol F, Kade S, et al. *Blood.* 2012;119(15):3578-3584. The negative prognostic impact with *SRSF2* mutations is from Thol F, Kade S, et al. *Blood.* 2012;119(15):3578-3584. Original illustration by DPS.

Key points (continued)

- contributes to the erythropoietic defect in del(5q) MDS and links del(5q) MDS to DBA, which is due to heterozygous germline 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 20 genes are known to harbor somatic mutations in patients with MDS. Those with IPSS-independent prognostic value include *EZH2*, *TP53*, *RUNX1*, *ASXL1*, and *NRAS*.

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. MDS arises from clonal expansion of multipotent or pluripotent HSCs or progenitor cells. Most studies of adults with MDS have shown that ineffective hematopoiesis, as opposed to a lack of hematopoietic activity as in 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 other molecular techniques indicate that the malignant clone in MDS includes CD34⁺ cells and differentiated myeloid, erythroid, and megakaryocytic cells. The B-cell lineage also may be involved, which may partially explain the immunologic disorders identified in some patients. Cell culture studies have shown reduced growth of multilineage colony-forming unit (CFU)—granulocyte-erythroid-monocyte-megakaryocyte progenitors (GEMM) 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 and might underlie the responses of some patients to pharmacologic doses of hematopoietic growth factors.

Experimental evidence 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 or of TNF α and its receptors. In marrow cultures, strategies that block TNF α -mediated signals, such as the use of anti-TNF α antibodies, 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. Conditional knockout of the *Dicer* gene in osteoprogenitor cells in mice induced development of an MDS-like syndrome, although the relevance of this for the clinical condition is unclear.

Key points (continued)

genome sequencing shows that most patients with MDS have at least five 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.

Hypoplastic MDS

About 10%–20% of patients with MDS have a hypocellular marrow resembling AA. Factors used to differentiate hypoplastic MDS from AA include morphologic evidence of dysplasia and an abnormal karyotype, both of which would be more consistent with MDS than AA. Given the subjectivity of the histologic criteria and the presence of transient chromosomal abnormalities in some patients with AA, however, differentiating these two disorders can be difficult. Although clinical characteristics and prognosis of hypoplastic MDS do not appear significantly different than the more typical normocellular or hypercellular MDS, a subgroup of hypoplastic MDS patients may respond to immunosuppressive therapy, as described in the following section.

Treatment of MDS

With the exception of allogeneic HSCT, no therapeutic options in MDS have demonstrated curative potential. However, three medications now have specific U.S. Food and Drug Administration (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) are expanding the roster of potentially eligible patients. Therefore, patients with MDS who are potentially candidates for transplantation should be evaluated early in the disease course by a physician with expertise in stem cell transplantation.

Goals of MDS therapy depend in part on the stage of disease and include symptom control, reduction of transfusion needs, delay of disease progression, and extension of survival. The IPSS classification and newer prognostic systems, such as the IPSS-R, allow clinicians to incorporate risk factors, median survival, and the risk of progression into therapeutic decisions.

Key points

- MDS is clonal disorder that arises in HSCs/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

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 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 some studies, lower-risk patients with MDS who have a ferritin >1000 ng/mL have experienced a poorer survival than lower risk MDS patients with a ferritin <1000 ng/mL, suggesting that transfusion-related iron overload also might be a contributing factor to poorer outcomes in transfusion-dependent patients.

Because the correlation between 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 ($R2^*$) magnetic resonance imaging, may be useful in determining which patients are the best candidates for iron chelation. Consideration should be given to initiation of iron chelation therapy with parenteral deferoxamine or oral deferasirox in 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. Platelet transfusions also may be necessary in some patients with MDS, but development of alloimmunization is problematic.

Hematopoietic growth factors

Hematopoietic growth factors have become an integral part of the treatment of MDS, despite the lack of a specific FDA-approved indication. Growth factors may reduce transfusion requirements by improving peripheral blood counts, and these agents are generally well tolerated.

Studies with recombinant erythropoiesis-stimulating agents (ESAs; epoetin and darbepoetin) demonstrated erythroid response rates in the range of 10% 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 RARS. No prospective studies have shown an alteration in survival with ESAs in MDS, although several retrospective studies suggest that ESAs may improve survival in MDS.

An 8- to 12-week trial of an ESA is appropriate for anemic patients with serum erythropoietin levels <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) and GM-CSF (sargramostim, molgramostim) have been evaluated in patients with MDS and increase the neutrophil count in up to 80%-90% of patients, which may help some patients who have recurrent infections. Some patients treated with G-CSF, however, just produce more functionally defective neutrophils and derive no benefit from myeloid growth factors, although the increased number of white cells may provide false reassurance to the patient and physician. 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, but survival was shorter in patients with 5%-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, 1-2 mg, rather than the standard 6 mg vial).

Thrombopoietin (TPO)-receptor agonists approved for use in immune thrombocytopenia, romiplostim and eltrombopag, are undergoing evaluation in patients with MDS. Romiplostim can improve the platelet count in many patients and reduce bleeding events, but a small number of patients experience an increase in blood or marrow blast proportion during romiplostim therapy. This is a concern 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 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 overexpress 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 antifibrinolytic 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.

Key points (continued)

- 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%-30% 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 RARS.
- ESAs are less effective in patients with high serum erythropoietin levels (≥ 500 U/L).
- TPO receptor agonists (thrombopoiesis-stimulating agents) can raise the platelet count in some patients with MDS, but they can increase the risk of AML progression and are not FDA approved for MDS.

Hypomethylating agents—DNA methyltransferase inhibitors

The cytidine residues in mammalian DNA can be methylated; DNA methylation is a dynamic process that affects transcription rates. Methylated cytidine residues cluster in so-called *cytosine-phosphate-guanine* (CpG) islands, which are located near the promoter regions of many genes. When these regions are hypermethylated, they are associated with gene silencing and represent a mechanism for regulating gene expression. DNA methyltransferase 1 (DNMT1) is the enzyme responsible for maintenance of cytidine methylation patterns, and the cytosine analogs azacitidine and decitabine can inhibit DNMT1, 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 to improve survival in high-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, low-dose cytarabine, or AML-like induction chemotherapy with infusional cytarabine and an anthracycline). The median survival time was 24 months in patients receiving azacitidine, versus 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. 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 4-6 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.

The most common adverse events for 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 maintain responses. Thus far, no therapy has been demonstrated to improve survival for patients with lower risk MDS.

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 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.

Deacetylase (DAC) inhibitors are another therapeutic strategy for MDS that also is based on the principle of epigenetic modification. DAC inhibitors are agents that maintain chromatin in a transcriptionally active state by inhibiting deacetylation of histone tails on chromatin; the acetylation state of cytoplasmic proteins also is modified during DAC inhibitor therapy, but their significance is unclear. *In vitro*, these agents lead to reversal of transcription repression and gene silencing and are synergistic in reactivating silenced genes when combined with hypomethylating agents. Several DAC inhibitors currently are under clinical investigation in MDS and AML, but in the first large randomized trial, E1905, the combination of azacitidine plus the DAC inhibitor entinostat (MS-275) was not superior to azacitidine monotherapy,

and combination therapy was associated with more adverse effects, such as fatigue and thrombocytopenia.

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 is usually not helpful. Such patients should be referred for HSCT or enrolled in clinical trials whenever feasible.

Immunomodulatory drugs

The drug thalidomide has multiple mechanisms of action, including alteration of immune cell subsets, inhibition of TNF α and other cytokines, inhibition of neoangiogenesis in the marrow, and inhibition of cereblon (a component of the ubiquitin ligase complex). When thalidomide was used in 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 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.

After phase I testing suggested a high response rate in del(5q) MDS, lenalidomide was tested in a phase II 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 II 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 II 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.

Lenalidomide at high doses (>10 mg/day) 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 less likely to achieve benefit from lenalidomide than those with a platelet count >50 \times 10⁹/L.

Immunotherapy

An autoreactive T-cell-mediated process may contribute to the pancytopenia in some patients with MDS. Several studies have demonstrated that treatment approaches analogous to immunosuppressive therapy of AA may be beneficial in MDS. Therapy with ATG or cyclosporine benefit some patients with lower risk disease (<10% blasts), especially those who are <60 years of age, lack transfusion dependence, and have either a normal karyotype or trisomy 8. Selection of patients most likely to respond to ATG or cyclosporine therapy remains challenging, because marrow hypocellularity and HLA-DR15 positivity have predicted response to immunosuppressive therapy in some studies but not in others.

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. 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.
- Some patients with MDS respond to ATG or cyclosporine 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) seem most likely to benefit.

Clinical case (continued)

The patient described earlier has an IPSS score of 1.5: 0.5 point for being pancytopenic, 0.5 point for an intermediate-risk karyotype, and 0.5 point for having a blast count between 5% and 10%. This places him in the IPSS intermediate-2 risk group, with an expected median survival of just over 1 year. The AZA-001 randomized trial demonstrated that IPSS intermediate-2-risk

Clinical case (continued)

and high-risk patients gain a median survival benefit of 9 months from azacitidine, which would be an appropriate therapy in this situation. Unfortunately, the patient is too old to consider allogeneic HSCT using currently available approaches.

Allogeneic HSCT

Allogeneic HSCT is the only definitive, curative therapy in MDS, but <10% of patients with MDS are eligible for myeloablative HSCT due to age and comorbidities. 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 and have a long-term disease-free survival of <10%, even with HSCT.

Allogeneic HSCT should be seriously considered for patients with higher risk MDS who have a matched sibling donor and a good performance status. Allogeneic transplantsations performed from matched-unrelated donors or umbilical cord blood are also a consideration in patients without a sibling donor. The use of reduced-intensity conditioning regimens may permit allogeneic HSCT in older individuals.

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 (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 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. 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.

HSCT is the treatment of choice for children with MDS. It is imperative to perform a DEB 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, 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 are a number of 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.

Key points

- Allogeneic HSCT remains the only routinely curative approach in MDS, and is an important consideration if the patient is young, otherwise healthy, and has an HLA-identical sibling or a closely matched unrelated donor. Cure rates overall are ~30%-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 17-9. 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 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 <500 U/L, epoetin or darbepoetin are recommended.

Current “standard” therapy for MDS		
Supportive care for all (transfusions and antimicrobials PRN, ?iron chelation)		
Lower risk MDS (assessed using IPSS, etc.)		
Cytopenia(s)	Disease feature	First-line therapy
Anemia only	Del (5q)	Lenalidomide
	No del(5q), sEPO <500	ESA ± G-CSF
	No del(5q), sEPO >500	?Immunotherapy
Neutropenia or thrombocytopenia or both		None established; observation, growth factors, aza/decit reasonable
Higher risk MDS		
Allogeneic SCT candidate?	Therapeutic approach	
Yes	Proceed to transplant ASAP; a hypomethylating agent (HMA) or cytotoxic chemotherapy may be useful as a “bridge”	
No	Azacitidine; decitabine as alternate	

Figure 17-9 Standard therapy for MDS. 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 (eg, IPSS low-risk or intermediate-1 risk groups) rather than for patients with higher risk disease. ASAP = as soon as possible; aza = azacitidine; decit = decitabine; ESA = erythropoiesis-stimulating agent; G-CSF = granulocyte colony stimulating factor; IPSS = International Prognostic Scoring System; MDS = myelodysplastic syndromes; NCCN = National Comprehensive Cancer Network; PRN = pro re nata (as the need arises); SCT = stem cell transplantation; sEPO = serum erythropoietin level. Partly based on 2012 NCCN guidelines; see <http://www.nccn.org>.

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 and are considered first-line therapy by many investigators. Patients with isolated thrombocytopenia may overlap with immune thrombocytopenia and may benefit from corticosteroids, romiplostim, or other immune thrombocytopenia-directed therapies. Immunosuppressive therapy, 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—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 pre-transplant 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 a well-designed study is available for which the patient is eligible.

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Acute myeloid leukemia
B. Douglas Smith and Lillian Sung

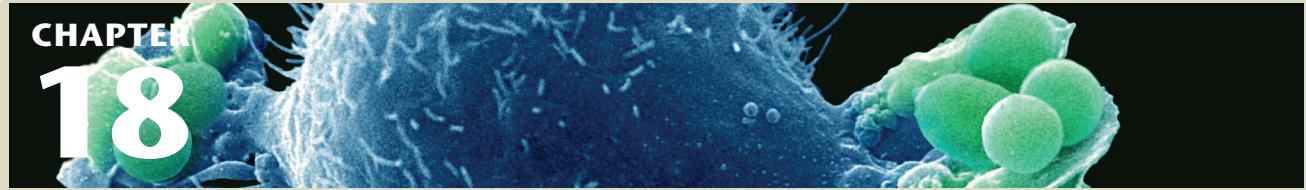
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CHAPTER
18



Acute myeloid leukemia

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Definition and epidemiology

Acute myeloid leukemia (AML) is a heterogeneous clonal stem cell malignancy in which immature hematopoietic cells proliferate and accumulate in bone marrow, peripheral blood, and other tissues. 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 approximately 13,000 new cases and 9,000 deaths in the United States in 2009. The annual incidence is approximately 3.5 per 100,000 and increases with age, with approximately a tenfold increased risk between ages 30 (1 case per 100,000) and 65 years (1 case per 10,000). The median age at diagnosis is 67 years, with ~6% of patients <20 years of age and 34% of patients 75 years or older. Overall survival in adults remains poor, with <50% 5-year survival in patients <45 years of age and <5% in patients >65 years of age at diagnosis. In children, overall survival has improved to ~60%.

Most cases of AML have no apparent cause. The most common known risk factor is previous exposure to radiation or chemotherapy, particularly topoisomerase II inhibitors and alkylating agents, which results in therapy-related AML (t-AML), and accounts for ~10%-20% of all AML cases. Those that arise after exposure to alkylating agents or radiation therapy have increased incidence with age, typically have a 5- to 10-year latency period, and frequently are associated with an antecedent therapy-related myelodysplastic syndrome (MDS) and unbalanced loss of genetic material involving chromosomes 5 or 7. t-AML associated

with exposure to topoisomerase II inhibitors encompasses 20%-30% of t-AML patients, has a shorter latency period of 1-5 years, is less often preceded by a myelodysplastic phase, and may be associated with balanced recurrent chromosomal translocations involving 11q23 (MLL) or 21q22 (RUNX1). Other environmental risk factors include exposure to benzene and ionizing radiation. Patients with inherited bone marrow failure syndromes (eg, Fanconi anemia, Shwachman-Diamond syndrome, severe congenital neutropenia), genetic disorders (eg, Down syndrome), and MDS and myeloproliferative disorders are also at increased risk of developing AML and have poor treatment outcomes.

Clinical manifestations

Patients with AML generally present with nonspecific signs and symptoms related to infiltration of the bone marrow and other organs with leukemic blasts, including 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/mL 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 they may be seen in all subtypes. Metabolic abnormalities related to tumor lysis syndrome also may be present, including hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia.

Conflict-of-interest disclosure: Dr. Smith declares no competing financial interest. Dr. Sung declares no competing financial interest.

Off-label drug use: Dr. Smith: not applicable. Dr. Sung: not applicable.

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-M7) on the basis of greater than or equal to 30% blasts, lineage commitment, and the degree of blast cell differentiation (Table 18-1). The FAB system has been largely replaced by the World Health Organization (WHO) classification, which was developed to incorporate epidemiology, clinical features, biology, immunophenotype, and genetics into the diagnostic criteria. The WHO has identified seven subgroups of AML (Table 18-2).

AML is now defined as greater than or equal to 20% myeloblasts, monoblasts or promonocytes, erythroblasts, or megakaryoblasts in the peripheral blood or bone marrow, except in patients with the following cytogenetic abnormalities,

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] [except in APL], CD34, CD117) and myeloid antigens (eg, CD13, CD33); complex composite immunophenotypes, including non-lineage-restricted lymphoid markers, also may be seen.

Prognostic 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

FAB subtype	Name	Adult AML patients (%)	Features
M0	Undifferentiated acute myeloblastic leukemia	5%-10%	MPO <3%
M1	Acute myeloblastic leukemia without maturation	15%-20%	MPO ≥3%, <10% maturation beyond blast stage
M2	Acute myeloblastic leukemia with maturation	25%-30%	MPO ≥3%, <10% maturation beyond blast stage
M3	Acute promyelocytic leukemia (APL)	10%-15%	≥30 blasts + hypergranular promyelocytes, strongly MPO or Sudan black B positive; microgranular variant (M3v) has inconspicuous granules, 15% of APL
M4	Acute myelomonocytic leukemia	10%-20%	>20% monocytes, NSE positive
M4 eos	Acute myelomonocytic leukemia with eosinophilia	5%	Abnormal marrow eosinophils, associated with inv(16) or t(16;16)
M5	Acute monocytic leukemia	10%-20%	M5a (poorly differentiated, monoblastic) M5b (differentiated, promonocytes, monocytes); strongly NSE positive
M6	Acute erythroid leukemia (erythroleukemia)	5%	Erythroblasts ≥50%, dyserythropoiesis, glycophorin A(+)
M7	Acute megakaryoblastic leukemia	5%	Associated with marrow fibrosis, CD41 or CD61 often positive

Table 18-1 French-American-British (FAB) classification of acute myeloid leukemia (AML).

Table 18-2 World Health Organization 2008 classification of acute myeloid leukemia (AML) and related myeloid neoplasms.

1. AML with recurrent genetic abnormalities
a. AML with balanced translocations/inversions
i. AML with t(8;21)(q22;q22); <i>RUNX1-RUNX1T1</i>
ii. AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>
iii. Acute promyelocytic leukemia with t(15;17)(q22;q12); <i>PML-RARα</i>
iv. AML with t(9;11)(p22;q23); <i>MLLT3-MLL</i>
v. AML with t(6;9)(p23;q34); <i>DEK-NUP214</i>
vi. AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); <i>RPN1-EVI1</i>
vii. AML (megakaryoblastic) with t(1;22)(p13;q13); <i>RBM15-MLK1</i>
b. AML with gene mutations
i. Provisional entity: AML with mutated <i>NPM1</i> (nucleophosmin)
ii. Provisional entity: AML with mutated <i>CEBPA</i> (CCAAT/enhancer binding protein A)
2. AML with myelodysplasia-related changes
3. Therapy-related myeloid neoplasms
4. AML, not otherwise specified
a. AML with minimal differentiation
b. AML without maturation
c. AML with maturation
d. Acute myelomonocytic leukemia
e. Acute monoblastic/monocytic leukemia
f. Acute erythroid leukemia
i. Pure erythroid
ii. Erythroleukemia, erythroid/myeloid
g. Acute megakaryoblastic leukemia
h. Acute basophilic leukemia
i. Acute panmyelosis with myelofibrosis
5. Myeloid sarcoma
6. Myeloid proliferations related to Down syndrome
a. Transient abnormal myelopoiesis
b. Myeloid leukemia associated with Down syndrome
7. Blastic plasmacytoid dendritic cell neoplasm

(t-AML), and the presence of an antecedent hematologic disorder (typically, MDS or myeloproliferative disorders). Patients >60 years, and especially those >75 years, have poor long-term survival because of both disease- and host-related factors, including increased expression of multidrug resistance genes, medical comorbidities, and poor performance status. White blood cell (WBC) count >50,000/mL at diagnosis is associated with increased risk of early death from hemorrhage or end-organ failure resulting from leukostasis.

Originally, the morphologic characteristics of the newly diagnosed disease was the critical determinant in prognostication. It now is evident, however, that chromosomal

(cytogenetic) and molecular abnormalities are the primary tools in best assigning prognosis for patients with newly diagnosed AML. It is imperative that the initial diagnostic workup include testing for the commonly described abnormalities, typically determined by bone marrow aspirate studies.

Acquired, nonrandom, clonal chromosomal abnormalities, including balanced translocations, inversions, deletions, monosomies, and trisomies may be found in up to 50% of patients with AML. The karyotype is considered complex when there are more than three abnormalities, and this can occur in 10%-20% of patients. Cytogenetic findings remain one of the most important prognostic tools and often are classified into favorable, intermediate, and unfavorable risk groups, but clinical study groups in AML assign certain abnormalities differently (Table 18-3). It is universally agreed, however, that patients with the t(15;17)(q22;q12-21) found in 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 normal karyotype disease (Figure 18-1). This is the largest cytogenetic subset of AML and without further ability to classify these patients, most generally fall into an intermediate-risk group. Yet, these intermediate-risk patients have variable outcomes with conventional treatment strategies, which may be explained by the underlying molecular heterogeneity associated with their disease. For example, 20%-30% of patients with AML have activating mutations of the receptor tyrosine kinase fms-like tyrosine kinase 3 (*FLT3*), and the internal tandem duplications (*FLT3-ITD*) within the juxtamembrane domain consistently are associated with inferior outcome in patients with cytogenetically normal AML. In addition, heterozygous mutations in exon 12 of the nucleophosmin member 1 (*NPM1*) gene have been found in 40%-60% of AML patients with a normal karyotype, and mutated *NPM1*, in conjunction with wild-type *FLT3*, is associated with a favorable prognosis. Finally, mutations of the CCAAT-enhancer binding protein A (*CEBPA*), a gene encoding a myeloid transcription factor important for normal granulopoiesis, also appear to be associated with favorable clinical outcomes.

Recent efforts to combine the information from cytogenetics and molecular changes have culminated in the evolution of the traditional risk groups into favorable, intermediate-1, intermediate-2, and adverse categories, which is a

Table 18-3 Variation in cytogenetic risk group classification across clinical trial groups.

	Original MRC	SWOG/ECOG	CALGB	GIMEMA/AML10	German AMLCG	HOVON/SAKK	Refined MRC
Favorable	t(15;17) t(8;21) inv(16)/t(16;16)	t(15;17) t(8;21) [lacking del(9q), complex ≥ 3 unre] abn] inv(16)/t(16;16)	t(15;17) t(8;21) inv(16)/t(16;16)	t(15;17) t(8;21) inv(16)/t(16;16)	t(15;17) t(8;21) alone inv/del(16) and lacking unfav abn	t(15;17) t(8;21) alone inv/del(16) inv(16)/t(16;16)	t(15;17) t(8;21)
Intermediate	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	Other noncomplex	+6,+8,-Y,del(12p)	Other noncomplex	-Y	Other noncomplex	Other noncomplex	Other noncomplex
Adverse	abn(3q) -5/del(5q) -7	abn(3q),(9q),(11q),(21q) abn(17p) -5/del(5q) complex [≥ 5 unre abn] Excluding those with favorable changes	inv(3)/t(3;3) -7 t(6;9) t(6;11) t(11;19) +8 complex [≥ 3 unre abn]	Other abn(3q) -5/del(5q) -7/del(7q) abn(11q23) del(12p) abn(17p) complex (≥ 3 unre abn)	inv(3)/t(3;3) -5/del(5q) -7/del(7q) abn(11q23) t(6;9) t(9;22) complex (≥ 3 unre abn)	abn(3q) -5/del(5q) -7/del(7q) abn(11q23) t(6;9) t(9;22) complex (≥ 3 unre abn)	abn(3q) -5/del(5q) -7/del(7q) abn(11q23) t(6;9) t(9;22) t(10;11)
			Excluding those with favorable changes		-17	abn(17p) with other changes Complex (>3 unre abn)	

abn = abnormal; add = addition; AMLCG = Acute Myeloid Leukemia Cooperative Group; CALGB = Cancer and Leukemia Group B; del = deletion; ECOG = Eastern Cooperative Oncology Group; GIMEMA = Gruppo Italiano Malattie e Matologiche dell'Adulto; HOVON/SAKK = Dutch-Belgian Hemato-Oncology Cooperative Group and the Swiss Group for Clinical Cancer Research; inv = inversion; MRC = medical research council; SWOG = Southwest Oncology Group; unfav abn = unfavorable abnormality; unre abn = unrelated abnormality.
References for the various classification systems can be found in Grimwade D. Impact of cytogenetics on clinical outcome in AML. In: Karp JE, ed. *Acute Myelogenous Leukemia*. Totowa, New Jersey: Humana Press; 2007:177-192.

The HOVON/SAKK cytogenetic classification was derived from the study by Cornelissen J, van Putten WL, Verdonck LF, et al. Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? *Blood*. 2007;109:3658-3666.
The revised MRC classification system was based on an analysis of 5,635 patients ages 16-59 years enrolled in the MRC AML10, AML12, and AML15 trials (Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in AML: determination of prognostic significance of rare recurring chromosomal abnormalities amongst 5,635 younger adults treated in the UK MRC trials. *Haematological Haematology*. 2009;94:217).

Table adapted with permission from Grimwade D, Hills RK. Independent prognostic factors for AML outcome. *Hematology Am Soc Hematol Educ Program*. 2009:385-395.

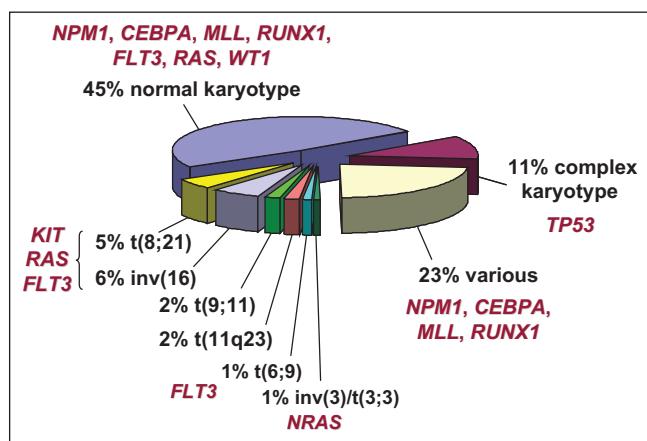


Figure 18-1 Major cytogenetic subgroups of AML (excluding acute promyelocytic leukemia) and associated gene mutations.

reminder that refining prognosis will continue to evolve as the impact of more targets is recognized.

Key points

- The most important prognostic indicators in AML are age, cytogenetics, and molecular genetics.
- 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 *NMP1* or *CEBPA* have a more favorable prognosis.

Treatment

Treatment for AML generally is divided into remission induction and postremission therapy. Standard remission induction regimens in the United States for all AML subtypes, excluding APL (see section on APL), almost always include 7 days of infusional cytarabine and 3 days of an anthracycline, commonly known as the "7+3" strategy. This strategy results in complete remission (CR) in 70%-80% of adults <60 years of age and 30%-50% of selected adults >60 years of age with a good performance status. 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 recently, daunorubicin 90 mg/m² has been shown, in large cooperative group trials, to be superior to 45 mg/m² even in selected patients >60 years of age. Many modifications to the standard "7+3" backbone have been attempted. Remission rates were similar or slightly improved when idarubicin or mitoxantrone was substituted for daunorubicin, but there was no convincing improvement in overall survival when equivalent doses were used. Randomized prospective trials also failed to demonstrate that induction with high-dose cytarabine (HiDAC) improved survival in most patient subgroups compared with standard induction. Similarly, addition of 6-thioguanine, etoposide, or dexamethasone to the anthracycline or cytarabine backbone did not improve overall survival. Finally, despite compelling scientific rationale, neither the addition of multidrug resistance modulators nor the addition of cytokines to chemotherapy (granulocyte-colony stimulating factor priming) has improved outcomes in AML to date. Trials combining targeted antibodies and *FLT3* inhibitors with chemotherapy are ongoing. Results for the targeted agent gemtuzumab ozogamicins have been conflicting to date but may benefit specific subgroups of patients.

Once remission has been achieved, further therapy is required to prevent relapse. Options include repeated courses of consolidation chemotherapy or hematopoietic stem cell transplantation (HSCT). Autologous HSCT permits escalation to myeloablative doses of chemotherapy, and allogeneic HSCT allows combination of myeloablative chemotherapy with a graft-versus-leukemia effect from the donor cells. Several studies have prospectively evaluated the role of intensive consolidation with HiDAC. The CALGB randomized patients in first remission to four courses of cytarabine using either a continuous infusion of 100 mg/m² for 5 days or a 3-hour infusion of 400 mg/m² or 3 g/m² twice daily on days 1, 3, and 5. Significant CNS toxicity was observed in patients >60 years old randomized to the high-dose arm, and thus, this regimen is not recommended for older patients. In patients <60 years old, there was a significant improvement in disease-free survival associated with the high-dose regimen, and this was most pronounced in patients with favorable cytogenetics, including t(8;21) and inv(16). Although it has become standard to offer at least two cycles of HiDAC at 1 to 3 g/m² to younger patients with AML, 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 three cycles of HiDAC consolidation were better than two cycles. Although it is clear that patients with CBF leukemias specifically benefit from HiDAC, some of these patients also have mutations in *KIT*, which are associated with an inferior outcome; clinical trials of

chemotherapy combined with tyrosine kinase inhibitors are ongoing in these patients. Consolidation chemotherapy, in general, has not been proven to be of benefit for patients >60 years old, but older patients able to tolerate additional treatment often are offered one or two cycles of 5 days of cytarabine combined with 2 days of an anthracycline after induction. Maintenance therapy 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 therapy improves outcomes.

Several studies of postremission 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%–45% at 3–5 years. There is no specific indication for its use in any prognostic subgroup.

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. It is, however, associated with significant morbidity and mortality. A recent comprehensive meta-analysis by Koreth et al. (2009) 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⁻*, and prospective trials using molecular and cytogenetic risk stratification are under way. 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 postremission therapy.
- The standard of care for induction for all AML subtypes in adults, excluding APL (FAB-M3), remains 3 days of an anthracycline combined with 7 days of cytarabine.
- Consolidation chemotherapy with two to four cycles of HiDAC is of particular benefit for patients <60 years old with favorable prognosis cytogenetics involving CBF [*t(8;21)* and *inv(16)*]; it is not routinely recommended for patients >60 years old.
- Allogeneic stem cell transplantation appears to be of benefit for AML patients in first remission who have intermediate- or poor-risk cytogenetics.
- Retrospective data suggest that allogeneic transplantation may be of benefit for patients with *FLT3-ITD⁺*.

Monitoring residual disease

Although morphologic methods remain the gold standard for determining the status of disease in AML, more sensitive immunologic and molecular methods for detecting the presence of minimal residual disease (MRD) are available. Leukemia-associated immunophenotypes can be identified as “signatures” for some patients with AML, and the presence of MRD as measured by immunophenotype has been shown to predict for disease relapse in some studies. The genetic abnormalities associated with a significant proportion of AML cases provide unique markers that can be used to monitor MRD. Polymerase chain reaction (PCR) offers a qualitative detection of abnormal gene rearrangements or fusion genes, whereas real-time PCR offers both the advantage of higher sensitivity and the possibility of quantification. MRD is now being used to risk stratify patients in current clinical trials. The optimal sensitivity for detection methods remains to be determined, and prospective clinical trials are required to assess whether additional postremission 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 of relapsed disease is poor, and these patients 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 up to a 50% chance of responding to a HiDAC-containing regimen, even if they had previous exposure to this agent. Examples of reinduction regimens include mitoxantrone with high-dose cytarabine and fludarabine, high-dose cytarabine and granulocyte colony-stimulating factor priming (FLAG). 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 short. The prognosis for patients who relapse after allogeneic transplantation is dismal.

Many categories of novel agents for AML are under investigation, including chemotherapeutics (eg, topoisomerase II inhibitors, purine nucleoside analogs), FLT3 inhibitors, DNA methyltransferase inhibitors, proteasome inhibitors, and farnesyltransferase inhibitors. In general, targeted agents have had limited single-agent activity in relapsed AML and may be more effective in combination with chemotherapy.

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, emerging data suggest that 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 transplants, are both feasible and effective in selected patients >60 years old. Many, if not most, older patients with AML fail to benefit from therapy because of lack of therapeutic efficacy, not intolerable toxicity. Novel therapies are clearly needed for this population, and there are many ongoing clinical trials with cytotoxics, antibodies, farnesyltransferase inhibitors, hypomethylating agents, and nonmyeloablative transplantations. Older AML patients should be encouraged to participate in clinical trials whenever possible.

Older patients with AML

Clinical case

An 82-year-old woman with a history of myocardial infarction, diabetes, and peripheral vascular disease presents with shortness of breath and is found to be pancytopenic. Bone marrow biopsy shows 40% myeloblasts with monosomy 7.

Most patients with AML are >60 years old, and their prognosis is dismal, with median survival times of only 8–12 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 supports. 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, relapse is almost certain, and <10% of patients are long-term survivors. Major cooperative group trials, which generally favor patients <75 years old with de novo AML and a good performance status, show 3- to 5-year overall survival rates of only 10%–20%. Many older patients are not offered any treatment for AML despite randomized data clearly

Acute promyelocytic leukemia

Clinical case

A 23-year-old Hispanic female presents with 2 weeks of dyspnea, bruising, and menorrhagia. Laboratory evaluation shows pancytopenia with elevated prothrombin and partial thromboplastin times and markedly decreased fibrinogen. Bone marrow aspiration shows intensely myeloperoxidase-positive promyelocytes and t(15;17).

APL (FAB-M3) is a clinically, cytogenetically, and prognostically distinct subtype of AML that accounts for ~5%–15% of all adult AML cases, with a higher incidence among Hispanics. It is the most curable form of AML in adults. Almost all leukemic cells from patients with APL have a balanced reciprocal translocation between chromosomes 15 and 17, which results in the fusion of the promyelocytic leukemia (*PML*) and retinoic acid receptor- α (*RAR\alpha*) genes, a *PML-RAR\alpha* fusion gene product, and disruption of normal differentiation. APL blasts contain granules with proteolytic enzymes, the release of which induces severe coagulopathy and fibrinolysis, predisposing patients to both hemorrhage and thrombosis.

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) may be found in most cases (Figure 18-2). Cases of microgranular APL have predominantly bi-lobed

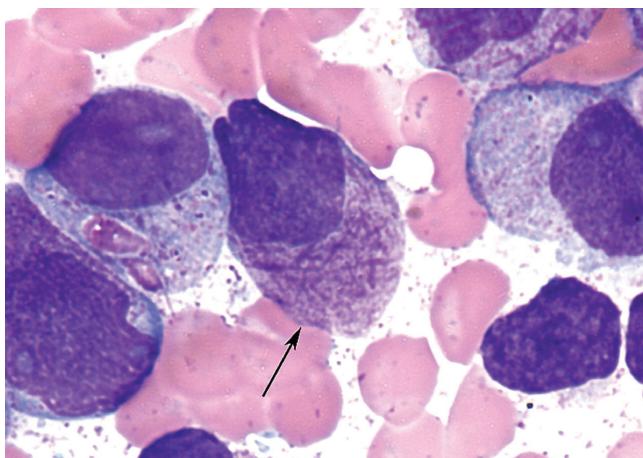


Figure 18-2 Faggot cell. From Maslak P. American Society of Hematology Image Bank, April 2008, #7-00039.

nuclei, are strongly myeloperoxidase positive, and often have a very high leukocyte count and doubling time. APL is characterized by low expression or absence of HLA-DR, CD34, CD117, and CD11b. The diagnosis is confirmed with cytogenetics, reverse transcriptase PCR (RT-PCR) for the *PML-RAR* fusion transcript, and fluorescence in situ hybridization with probes for *PML* and *RAR α* , or anti-*PML* antibodies.

APL promyelocytes have the unique ability to undergo differentiation with exposure to all-*trans* retinoic acid (ATRA). Detection of t(15;17) or the underlying *PML-RAR α* rearrangement is predictive of response to ATRA in virtually 100% of cases. Some infrequent APL variants, such as t(11;17) (q23;q21) with *ZBTB16-RARA* and cases with *STAT5B-RARA* fusions, are resistant to ATRA. It is crucial that ATRA is started as soon as the diagnosis of APL is suspected, before pathologic confirmation. 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%-80% of patients in many series. Primary resistance to chemotherapy is virtually nonexistent. Three prospective trials have compared ATRA with or without chemotherapy, with chemotherapy alone in APL induction; all showed benefits in event-free survival, disease-free survival, and overall survival with the addition of ATRA. Thus, standard treatment generally includes anthracycline-based chemotherapy plus ATRA. 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/m² for 3 days or idarubicin 12 mg/m² 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. The role of infusional cytarabine during induction is not clear, but patients presenting with a WBC count \geq 10,000/mL may benefit from intermediate-dose

cytarabine or HiDAC during either induction or consolidation. Some protocols for high-risk patients have also incorporated prophylactic intrathecal chemotherapy. The role of maintenance therapy is also controversial in APL, but ATRA with or without 6-mercaptopurine and methotrexate frequently is offered to patients in CR. The optimal combination and duration of maintenance have not been defined.

Despite the success of ATRA-based regimens, there is still an early mortality of ~10% in APL patients, primarily because of hemorrhagic complications. Predictors of early death resulting from hemorrhage include WBC count at presentation, abnormal creatinine, peripheral blast count, presence of coagulopathy, and age. Also, patients must be closely monitored for the development of APL differentiation syndrome, a potentially fatal constellation of findings, including interstitial pulmonary infiltrates, hypoxemia, respiratory distress, fluid retention, weight gain, pleural or pericardial effusions, and sometimes renal failure. Rapid administration of dexamethasone 10 mg twice daily for at least 3 days at the earliest manifestations of the syndrome can be lifesaving.

The persistence or reappearance of *PML-RAR α* fusion gene transcripts in patients with APL is highly predictive of clinical relapse, and frequent monitoring approximately every 3 months by RT-PCR, is considered standard of care. Relapsed disease can be treated effectively with arsenic trioxide, which causes differentiation and apoptosis of APL cells, alone or in combination with ATRA. Autologous stem cell transplantation can be considered for patients in second remission if the stem cells are negative for *PML-RAR α* . Allogeneic stem cell transplantation generally is not recommended for patients with APL but may be considered for relapsed patients.

Arsenic trioxide may be the single most active agent in APL and appears to offer a survival benefit when given as consolidation for newly diagnosed patients. It also produces high rates of durable CR when combined with ATRA in newly diagnosed patients. Treatment with the combination of arsenic and ATRA has allowed reduction or elimination of chemotherapy for selected newly diagnosed patients with APL and frequently is offered to older patients who cannot tolerate conventional chemotherapy.

Key points

- APL is a unique subtype of AML that is exquisitely sensitive to ATRA, anthracyclines, and arsenic trioxide.
- ATRA should be started immediately if the diagnosis of APL is suspected.
- Cure rates are high in APL.
- APL may be complicated by a life-threatening coagulopathy or differentiation syndrome.
- APL differentiation syndrome should be treated promptly with dexamethasone 10 mg twice daily for at least 3 days.

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 <1 year of age. Poor-prognosis cytogenetics are less frequent in children, and within the pediatric spectrum, age is not a critical prognostic indicator, except for children with Down syndrome. Children may tolerate intensive chemotherapy better than adults, and this 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% irrespective of response to the first cycle of induction, whereas children with adverse cytogenetics or poor response to the first cycle of induction therapy with >15% residual blasts have an overall survival rate of only 15%.

Children with Down syndrome have a 46- to 83-fold increased risk of AML and are generally younger than other pediatric AML patients. AML associated with Down syndrome tends to be classified as FAB-M7 (acute megakaryoblastic leukemia [AMKL]), and *GATA1* mutations have been described in the leukemic blasts. AMKL in Down syndrome may be preceded by transient myeloproliferative disorder (TMD), a condition unique to children with Down syndrome. 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%-20% of cases. For those who survive, ~20%-30% will later develop AMKL. Children with Down syndrome and AML who are <2-4 years of age have better

prognosis compared with both non-Down syndrome AML and Down syndrome AML patients >4 years of age at diagnosis. This superior prognosis may be related to enhanced sensitivity of the leukemic blast to cytarabine. Children with Down syndrome have greater toxicities with treatment and usually are not offered HSCT in first remission.

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Daniel J. DeAngelo and Ching-Hon Pui

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CHAPTER
19



Acute lymphoblastic leukemia and lymphoblastic lymphoma

Daniel J. DeAngelo and Ching-Hon Pui

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common leukemia in children (representing 23% of all cancer diagnoses and 76% of leukemias among children <15 years of age) but accounts for only 20% of adult acute leukemia. The prognosis for both adult and especially childhood ALL has improved substantially in recent years with the use of risk-directed induction-consolidation-continuation (maintenance) regimens that include central nervous system (CNS) prophylaxis. In children, treatment now results in complete remission (CR) rates of 97%-99%, 5-year event-free survival rates of 75%-87%, and 5-year survival rates of 90%-94%. The use of similar treatment regimens in adults with ALL also has improved the prognosis, with CR rates of 65%-90% and 5-year survival rates of 25%-50%. The less favorable prognosis for adults with ALL is related to several factors, including a much higher frequency of poor-risk prognostic factors based on disease biology, comorbidities associated with older age that impair the ability to tolerate intensive multiagent chemotherapeutic regimens that have been used successfully in children, subtle differences in the treatment regimens used by medical oncologists treating adults, and treatment adherence.

Classification and diagnosis of ALL

The French-American-British (FAB) morphologic classification of ALL was based largely on morphology and contained little prognostic or therapeutic information that might help to guide treatment choice. The World Health Organization (WHO) classification was revised in 2008 and has changed the classification to reflect increased understanding of the biology and molecular pathogenesis of the diseases. In addition to discarding the FAB terms, 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 new classification further subdivides the precursor B-cell ALL cases by recurring molecular-cytogenetic abnormalities to provide prognostic and therapeutic information and to facilitate the implementation of specific molecularly targeted therapies. Burkitt lymphoma/leukemia is the one subset of ALL that is classified as a mature B-lymphoid neoplasm.

Examination of a bone marrow aspirate is preferable to blood for diagnosis of ALL because as many as 10% of patients lack circulating blasts at the time of diagnosis and because bone marrow cells are better than blood cells for genetic studies. Fibrosis or tightly packed marrow occasionally can lead to difficulties with marrow aspiration that necessitate a biopsy to make the diagnosis. In patients with marrow necrosis, multiple marrow aspirations are sometimes needed to obtain diagnostic tissue.

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Immunophenotyping

Because leukemic lymphoblasts lack specific morphologic and cytochemical features, immunophenotyping by flow cytometry and genetic analyses are essential for diagnosis. Most leukocyte antigens lack lineage specificity; hence, a panel of antibodies is needed to establish the diagnosis and to distinguish among the different immunologic subclasses of leukemic cells. In general, the panel includes antibodies to at least one very sensitive marker for each hematopoietic and lymphoid lineage (CD19 for B-lineage cells, CD7 for T-lineage cells, and CD13 or CD33 for myeloid cells) and antibodies to a relatively specific marker (cytoplasmic CD79a and CD22 for B-lineage cells, cytoplasmic CD3 for T-lineage cells, and CD20 and surface immunoglobulin for mature B cells).

Although ALL can be classified according to the normal sequential stages of normal T-cell and B-cell development, it is therapeutically useful only to distinguish among T-cell ALL, B-lymphoblastic ALL, and Burkitt ALL. Typically, B-lymphoblastic ALL cases are terminal deoxynucleotidyl transferase (TdT) positive, human leukocyte antigen (HLA)-DR positive, and almost always positive for CD19 and CD79a; CD10 and CD22 are positive in most cases. The lymphoblasts in T-cell ALL are TdT positive and most often express CD7 and cytoplasmic CD3. There is a distinct subset of T-cell ALL termed early T-cell precursor ALL, with immunologic markers and gene expression profile reminiscent of double-negative 1 thymocyte that retains the ability to differentiate into T-cell and myeloid, but not B-cell, lineages. These cases are associated with a dismal treatment outcome with chemotherapy. The mutational spectrum of this subtype recapitulates that of acute myeloid leukemia by whole genome sequencing, and global transcriptional profiling is similar to that of normal hematopoietic stem cell and myeloid leukemia, suggesting that this is a stem cell disease. The prevalence of mutations in genes regulating cytokine receptor and Ras signaling, as well as chromatin modification, suggests that myeloid-directed therapy or epigenetic therapy might be useful for early T-cell precursor ALL. The mature B-cell ALL, Burkitt ALL, has a unique immunophenotype with expression of surface immunoglobulin, has a strong expression of CD20, is negative for TdT expression, and also has distinctive morphologic and cytogenetic features. These ALLs are associated with chromosome translocations involving the *c-MYC* proto-oncogene on chromosome 8. The distribution of the immunophenotypic subsets differs slightly between adult and pediatric ALL. T-cell ALL accounts for 25% of adult ALL and 10%-15% of pediatric ALL cases, Burkitt ALL accounts for ~2%-5% of adult and pediatric ALL cases, and B-lymphoblastic ALL accounts for the remaining cases. There are also racial and ethnic differences in the distribution, with T-cell ALL accounting for 10%-12% of white and accounting for 25% of black children with ALL.

Myeloid-associated antigens may be expressed on otherwise-typical lymphoblasts. The pattern of myeloid-associated antigen expression is correlated with certain genetic features of blast cells. CD15, CD33, and CD65 are expressed in ALL patients with a rearranged *MLL* gene, and CD13 and CD33 are expressed in patients with the *ETV6-RUNX1* (also known as *TEL-AML1*) fusion. A subset of patients coexpress both lymphoid and myeloid markers but do not cluster with T-cell, B-lymphoblastic, or acute myeloid leukemia in gene expression profiling. These patients may not respond to myeloid-directed therapy but may attain remission with ALL-directed induction treatment. The presence of myeloid-associated antigens lacks prognostic significance but can be useful in immunologic monitoring of patients for minimal residual leukemia. A summary of CD markers and specific immunophenotypic techniques and findings in ALL is found in Chapter 10.

Cytogenetics

ALL arises from a lymphoid progenitor cell that has sustained multiple specific genetic damages that lead to malignant transformation and proliferation. Thus, genetic classification of blast cells is expected to yield more relevant biologic information than that obtained by other means. More than 75% of adult and childhood cases can be readily classified into prognostically or therapeutically relevant subgroups based on the modal chromosome number (or DNA content estimated by flow cytometry), and specific chromosomal rearrangements. Table 19-1 lists selected cytogenetic and molecular genetic abnormalities with prognostic and therapeutic relevance. Increasingly, as described in later sections on therapy, treatment strategies are being specifically tailored to the different genetic subsets of ALL.

According to the modal chromosomal number, ALL can be classified into several ploidy subgroups. Hyperdiploidy >50 chromosomes, which is seen in approximately 25% of childhood cases and in 6%-7% of adult cases, is associated with a favorable prognosis in childhood ALL and in some studies of adult ALL, and may reflect an increased cellular accumulation of methotrexate and its polyglutamates, an increased sensitivity to antimetabolites, and a marked propensity of these cells to undergo apoptosis. The outcome of hyperdiploid cases in adults is not comparable to the excellent outcome of childhood cases and hyperdiploidy has lacked favorable prognosis in some adult studies. By contrast, hypodiploidy <44 chromosomes, especially near haploid (24-31 chromosomes) and low-hypodiploidy (32-39 chromosomes), consistently is associated with an adverse prognosis in both children and adults with ALL. Flow cytometric determination of cellular DNA content is a useful adjunct to cytogenetic analysis because it is automated,

Table 19-1 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
Hypodiploid <44 chromosomes	2	1	10-20 at 3 years	30-40 at 3 years	
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
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	80-90 at 3 years	ABL1 tyrosine kinase inhibitors (imatinib/dasatinib)
t(4;11)(q21;q23)/MLL-AF4 fusion	3-7	2	10-20 at 3 years	30-40 at 5 years	FLT3 inhibitors (PKC412/CEP-701)
BCR-ABL1-like/IKZF1 alterations	Unknown	15-20	Unknown	40-50 at 5 years	Tyrosine kinase inhibitors in some cases
iAMP21	Unknown	2	Unknown	60-70 at 5 years	Intensive glucocorticoid, asparaginase and vincristine
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)

ALL = acute lymphoblastic leukemia; iAMP21 = intrachromosomal amplification of chromosome 21.

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 sometimes can identify a small but drug-resistant subpopulation of near-haploid or low-hypodiploid cells that may have been missed by standard cytogenetic analysis.

Specific reciprocal translocations have important biologic and clinical significance. Some translocations can mobilize the promoter-enhancer element of the immunoglobulin heavy- or light-chain gene or the T-cell antigen receptor β/γ or α/δ gene to sites adjacent to a variety of transcription factor genes and can result in the overexpression of the transcription factor. An example of this type of translocation occurs in Burkitt ALL, in which the transcription factor *c-MYC* is translocated to the promoter-enhancer element of the immunoglobulin heavy- or light-chain and, consequently, is expressed aberrantly. More often, the genetic rearrangements result from the fusion of two genes encoding different transcription factors. 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 *MLL* gene on chromosome 11q23, the most

common of which is t(4;11), which results in the creation of the *MLL-AF4* (alias *AFF1*) 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 t(9;22) results in the *BCR-ABL1* fusion gene and causes constitutive activation of the ABL tyrosine kinase, which is directly linked to disease pathogenesis.

Molecular genetics

In addition to cytogenetic analysis, there are compelling reasons to perform molecular genetic studies. First, molecular analyses can identify several important submicroscopic genetic alterations not visible by standard karyotyping procedures, such as the *ETV6-RUNX1* (also known as *TEL-AML1*) fusion, intrachromosomal amplification of chromosome 21, deletions of tumor suppressor genes, and mutations of proto-oncogenes. Second, cases with clinically important genetic rearrangements can be missed because of technical errors (eg, karyotyping residual normal metaphase cells rather than leukemic metaphase cells). Hence, fluorescence in situ

hybridization (FISH) and reverse-transcriptase polymerase chain reaction (RT-PCR) assays are used frequently.

More recently, the application of microarray-based genomewide 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 many specific genetic alterations. As a result, virtually all patients with ALL can be classified according to specific genetic abnormality to date. These studies also provided insight into the complex interactions of multiple genetic alterations in leukemogenesis and response to therapy. T-cell ALL cases can be classified into several distinct genetic subgroups that correspond to discrete T-cell development stages: *HOX11L2*, *LYL1* plus *LMO2*, *TAL1* plus *LMO1* or *LMO2*, *HOX11*, and *MLL-ENL*. Whereas *HOX11L2* generally confers a poor outcome, *HOX11* and *MLL-ENL* are associated with a favorable outcome. Among many other mutations in T-cell ALL, *NOTCH1* or *FBXW7* mutations are associated with a favorable prognosis in childhood ALL, and the *NUP214-ABL1* fusion is responsive to tyrosine kinase inhibition. In an adult study with small number of patients, *NOTCH1* and *FBXW7* mutations failed to correlate with treatment outcome.

In B-lymphoblastic ALL with Philadelphia chromosome and *BCR-ABL1* fusion, *IKZF1* is deleted in both pediatric and adult cases (75%-85%). Of interest, a subgroup of Philadelphia chromosome-negative B-cell precursor ALL with *IKZF1* deletion (*BCR-ABL1*-like ALL) occurs in as many as 7%-9% of children with ALL, has a genetic expression profile similar to that of cases with *BCR-ABL1* fusion, and also has poor prognosis with conventional treatment. A recent transcriptome sequencing study of 12 precursor B-cell ALL cases (with whole genome sequencing study in two cases) identified structural alterations and mutations activating kinase and

cytokine receptor signaling in all cases studied. Importantly, fusion transcripts that would be responsive to tyrosine kinase inhibitors (TKIs) were identified in several cases. Another subgroup is characterized by increased *CRLF2* expression and occurs in 5%-7% of children with precursor B-cell ALL and, remarkably, in ~50% of the cases with Down syndrome. Many of these cases have cryptic translocations involving a tyrosine kinase gene (eg, *JAK*) and probably require more intensive therapy because this subtype is associated with a poor outcome. Among children with hypodiploid ALL, near-haploid ALL cases frequently have alterations targeting receptor tyrosine kinase signaling and Ras signaling (71%), and low-hypodiploid cases are characterized by alterations in *TP53* (91%) that commonly present in normal cells and may be inherited. Both of these two hypodiploid subtypes are responsive to P13K inhibitors in preclinical models.

Recent genome-wide association studies have identified germline single-nucleotide polymorphisms of several genes that are strongly associated with ALL susceptibility, racial disparities in the incidence, and treatment outcome. Hence, inherited genetic variations not only affect the development of ALL but also the response to treatment.

Prognostic factors

Of the many variables that influence prognosis, genetic subsets, initial white blood cell (WBC) count, age at diagnosis, and early treatment response are the most important. Although improved treatment has abolished the prognostic strength of many prognostic indicators in pediatric ALL, even so-called low-risk patients need a certain degree of treatment intensification to avoid unacceptable rates of relapse. Table 19-2 lists the prognostic factors in adults and children that may be used for risk stratification in current clinical trials.

Table 19-2 Prognostic factors used for risk stratification.

Prognostic factors	Favorable	Adverse
Adult		
Age (years)	<35	>60
Leukocyte count ($3 \times 10^9/L$)	<30 for B-cell	>100 for T-cell
Immunophenotype	Thymic T-ALL	Early T-cell precursor
Genotype	—	<i>BCR-ABL1</i> ; <i>MLL-AF4</i> ; Hypodiploidy <44;
Minimal residual disease after induction	<0.01%	>0.01%
Pediatric		
Age (years)	1 to 9	<1 or >10
Leukocyte count ($\times 10^9/L$)	<50	>50
Immunophenotype	B-lymphoblastic	Early T-cell precursor
Genotype	Hyperdiploidy >50; <i>ETV6-RUNX1</i>	Hypodiploidy <44; <i>MLL-AF4</i> , <i>IKZF1</i> deletions or mutations
Minimal residual disease after induction	<0.01%	>1%

Clinical prognostic factors

Children ages 1-9 years have a better outcome than either infants or adolescents, who, in turn, fare significantly better than adults with ALL. Among adults, the outcome of therapy worsens with increasing age. Leukocyte count is a continuous variable, with increasing counts conferring a poorer outcome. In childhood ALL, there is a general agreement of using 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 little prognostic value in T-cell ALL. In adult ALL, age <35 years and leukocyte count $<30 \times 10^9/L$ are considered favorable prognostic indicators, and leukocyte count $>100 \times 10^9/L$ is considered a poor prognostic feature for T-cell ALL in some studies.

Cytogenetic and molecular genetic prognostic factors

The prognostic impact of age and, to a lesser extent, leukocyte count can be explained by their association with specific genetic abnormalities. For example, there is a preponderance of cases with favorable genetic abnormalities of hyperdiploidy >50 chromosomes or *ETV6-RUNX1* in patients ages 1-9 years. *ETV6-RUNX1* mutations are rare in adults. Adverse genetic abnormalities such as *MLL* rearrangements occur in 70%-80% of infant cases. The Philadelphia chromosome (Ph+) occurs in 25%-30% of adult patients, and the incidence of Ph+ ALL increases with increasing age. Approximately 50% of cases of B-lymphoblastic ALL in patients >60 years of age are Ph+.

Although many genetic abnormalities are associated with clinical outcome (Table 19-1), there is no consensus on the specific genotypes used for treatment stratification. The Children's Oncology Group also uses trisomy of chromosomes 4, 10, and 17 (triple trisomy) as a favorable prognostic factor in its clinical trials. There is clinical heterogeneity within each specific genetic subtype. For example, among patients with Ph+ ALL treated without ABL1 TKI, age <15 years, leukocyte count $<25 \times 10^9/L$, and good early treatment response are independent favorable factors (Aricó et al., 2010). In adults, Ph+ ALL is associated with not only a high initial leukocyte count but also a dismal prognosis with standard chemotherapeutic regimens. Treatment intensification with allogeneic stem cell transplantation (allo-SCT) in first remission remains a standard curative approach for these adults. In patients with *MLL-AF4* fusion, infants and adults have a worse prognosis than children. The basis of these differences may be related to some combination of secondary genetic events, the developmental stage of the target cell undergoing malignant transformation, and the

pharmacogenetic or pharmacokinetic features of the patient. In addition to t(4;11), the *MLL* gene is involved in a number of other translocations in ALL (eg, t[11;19], t[9;11]). Because of their poor prognosis, allogeneic transplantation in first CR (CR1) is recommended for adult patients with translocations involving the *MLL* gene.

The clinical significance of many prognostic factors changes with improvements in treatment. For example, the outcome for patients with Ph+ ALL has improved substantially with the addition of TKIs to treatment, which is described in detail later. In fact, children with this genotype who are treated with intensive chemotherapy and imatinib (without allo-SCT) have a 3-year event-free survival rate of 88%. Another recent study confirmed that imatinib in combination with intensive chemotherapy is well tolerated and beneficial for children with Ph+ ALL. If the favorable outcome is confirmed with longer follow-up, Ph+ may join a long list of other factors such as male sex, African American ethnicity, and older adolescent age-group that have lost their adverse prognostic impact with improved treatment in childhood ALL. The recent finding of adverse prognosis of CD20 expression in adult ALL, albeit not childhood ALL, may have therapeutic implications as a result of the availability of the anti-CD20 monoclonal antibody rituximab.

Minimal residual disease detection

A useful adjunct in risk assessment is the response to early treatment, which is measured by the rate of clearance of leukemic cells from the blood or bone marrow. This measure accounts for the drug sensitivity or resistance of leukemic cells and the pharmacodynamics of the drugs, which are affected by the pharmacogenetics of the host. Flow cytometric profiling of aberrant immunophenotypes and PCR amplification of fusion transcripts or antigen-receptor genes, which are at least 100-fold more sensitive than conventional morphologic determinations, have allowed minimal residual disease (MRD) to be detected at very low levels (0.01% or lower), providing a useful means to identify patients at very low or high risk of relapse. MRD can be measured by these two techniques in nearly all patients and has become a crucial factor for risk stratification in childhood ALL. Recently, a new deep-sequencing method, which is even more sensitive and has universal applicability, has been developed. Patients who achieve molecular or immunologic remission ($<0.01\%$) after conventional remission induction have an excellent outcome and can continue treatment for standard (or low-risk) ALL. Patients with 1% or more leukemic cells after remission induction, especially those who fail to achieve clinical remission ($\geq 5\%$ leukemic cells in bone marrow) may become a candidate for allo-SCT,

with the exception of patients 1–6 years of age with hyperdiploidy >50 or *ETV6-RUNX1* who should continue treatment with intensive chemotherapy rather than transplantation. MRD detection after achievement of morphologic remission in adults with ALL has been associated with a significantly worse prognosis. Monitoring of MRD can be used for early detection of impending relapse and hence for early treatment intervention. Finally, MRD level is a strong predictor of treatment outcome at the time of second remission and before allo-SCT for relapsed leukemia in both pediatric and adult ALL.

MRD measurement is now used to improve risk stratification and to allocate patients to allo-SCT in most pediatric trials and in some adult clinical trials. MRD detection, either before or after allo-SCT, in adults with Ph+ ALL has been associated with a lower disease-free survival (DFS). On the basis of these insights, some investigators are examining the efficacy of posttransplantation therapy with imatinib or other targeted TKIs to eradicate MRD and improve posttransplantation progression-free survival.

Treatment of ALL

Supportive care

Optimal management of patients with ALL requires careful attention to supportive care. Hyperuricemia and hyperphosphatemia with secondary hypocalcemia frequently are encountered at diagnosis, even before chemotherapy is initiated, especially in patients with high leukemic cell burden and those with T-cell or mature B-cell ALL. Patients should be given intravenous fluids; allopurinol or rasburicase (recombinant urate oxidase) should be given to patients at high risk of tumor lysis syndrome to treat or prevent hyperuricemia; and a phosphate binder, such as aluminum hydroxide, calcium acetate or carbonate (if the serum calcium concentration is low), lanthanum carbonate, or sevelamer, should be given to treat or prevent hyperphosphatemia. Infections are common in febrile patients with newly diagnosed ALL. Therefore, any patient presenting with fever, especially those with neutropenia, should be given broad-spectrum antibiotics until infection is excluded. Usually, all patients with ALL are given trimethoprim-sulfamethoxazole, atovaquone, or pentamidine as prophylactic therapy for *Pneumocystis carinii* (*Pneumocystis jiroveci*) pneumonia. Some pediatric and many adult trials also recommend some form of antibacterial, antiviral, and antifungal prophylaxis in patients with severe leukopenia during the active phases of treatment. The use of hematopoietic growth factors for adults with ALL has been found to be safe and reduces the number of induction deaths. These studies are reviewed later in the treatment section of adult ALL. All blood products

should be irradiated to prevent transfusion-associated graft-versus-host disease. Other important supportive care measures include the use of indwelling catheters, amelioration of nausea and vomiting, pain control, and continuous psychosocial support for the patient and family.

Treatment of Burkitt lymphoma/leukemia in children and adults

The outcome for both children and adults with Burkitt lymphoma/leukemia has improved dramatically during the past decade. The improved outcomes have resulted from the use of fractionated high doses of alkylating 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 4–6 months. 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 often has been incorporated into treatment regimens. Because of an extremely high predisposition to CNS involvement in 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 typically is omitted and 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 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, ongoing studies have incorporated the anti-CD20 monoclonal antibody rituximab into frontline regimens in an attempt to further improve outcome. The data from these trials appear very promising, with survival rates of ~80%. The addition of rituximab to frontline therapies for Burkitt lymphoma/leukemia also is being tested in children.

Treatment of B-lymphoblastic and T-cell ALL in children

Although risk-directed therapy is a standard therapeutic strategy for childhood ALL, there is no consensus on the risk criteria and the terminology for defining prognostic subgroups. Usually, childhood ALL cases are divided into low-(standard-) risk, high- (intermediate- or average-) risk, and very high-risk groups, although the U.S. Children’s Oncology

Group advocates four categories, including a very low-risk group. Infants often are treated with a separate regimen. Treatment typically consists of a remission induction phase, an intensification (consolidation) phase, and prolonged continuation therapy to eradicate residual disease. 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 typically includes a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine, and asparaginase. Children with high- or very high-risk ALL receive one or more additional drugs, including an anthracycline and cyclophosphamide. Intensive induction can lead to increased early morbidity and mortality. In two studies, improved outcomes were noted with intensification with dexamethasone ($10 \text{ mg/m}^2/\text{day}$) in lieu of prednisone ($60 \text{ mg/m}^2/\text{day}$) for children with T-cell ALL and a good response to 7 days of upfront prednisone treatment, and in children <10 years of age with B-lymphoblastic ALL. Dexamethasone at a dose of 10 mg/m^2 is not recommended for remission induction in children 10 years of age or older with B-lymphoblastic ALL because of high rates of toxicity and toxic death associated with the treatment in this age-group. To this end, intensification of induction is not necessary for children with standard-risk ALL, particularly if they receive postinduction intensification therapy.

The efficacy of prednisone and dexamethasone is dose dependent. Although both drugs yielded comparable results when given equivalent doses, dexamethasone still appears to yield improved CNS control and is used preferentially in postremission therapy in current clinical trials. 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 for children in the United States and increasingly is being used in other clinical trials around the world. As a result, native *E. coli* asparaginase was no longer commercially available in the United States after 2012. Because antibodies to *E. coli* asparaginase cross-react with PEG-asparaginase, patients with allergic reactions to either form of the asparaginase should be treated with the product

derived from *Erwinia chrysanthemi*. Antibodies can develop against polyethylene glycol and adversely affect the efficacy of the drug; however, patients with antibodies against polyethylene glycol can be treated with native asparaginase. Asparaginase treatment preceded by a dexamethasone pulse or given without interruption are associated with a decreased risk of allergic reactions. Individualized dosing of asparaginase based on serum asparaginase activity and switching to an alternate form of asparaginase in the presence of "silent inactivation" may improve outcome. Of the various anthracyclines given to patients with ALL, none has proved superior to any other; however, daunorubicin is used most commonly.

Intensification (consolidation) therapy

When normal hematopoiesis is restored, patients in remission become candidates for intensification therapy. Although there is no dispute on the importance of this treatment, there is no consensus on the best regimen and duration of treatment. More commonly used regimens include high-dose methotrexate with mercaptopurine, or a combination of dexamethasone, vincristine, asparaginase, mercaptopurine, and doxorubicin. This phase of therapy has improved outcome, even for patients with low-risk ALL. Patients with *ETV6-RUNX1* have an especially good outcome in clinical trials featuring intensive postremission treatment with glucocorticoids, vincristine, and asparaginase. High-dose methotrexate (5 g/m^2) is associated with improved outcome in T-cell ALL, whereas lower doses appear to be sufficient for low-risk B-cell ALL.

Delayed intensification (or reinduction), first introduced by investigators of the Berlin-Frankfurt-Münster consortium, is a widely used approach consisting of a repetition of the first remission induction therapy 3 months after the end of remission induction. Investigators at the Children's Cancer Group reported that double-delayed intensification improved patient outcome in patients with intermediate-risk ALL. Although extended and stronger intensification therapy with asparaginase, methotrexate, and vincristine was shown to significantly improve outcome for children and adolescents with high-risk ALL and slow response to initial induction therapy, recent studies demonstrated that intensification treatment for 6 months is as effective as 10 months of such therapy for high-risk patients with a rapid early response. Two recent studies have shown that double-delayed intensification is not necessary for low-risk or even high-risk patients if they had a rapid early response to remission induction therapy. Notably, delayed intensification in both studies was given rather late in the treatment course (week 48 and week 32 from diagnosis, respectively).

Maintenance (continuation) therapy

A combination of methotrexate administered weekly and mercaptopurine administered daily constitutes the usual continuation regimen for ALL. In the past, boys were treated with a longer duration of continuation therapy than girls because male sex was 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 clinical trials. Accumulation of higher intracellular concentrations of the active metabolites of methotrexate and mercaptopurine and administration of this combination to the limits of tolerance (as indicated by low leukocyte counts) have 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 and $1.5 \times 10^9/L$ to ensure adequate dose-intensity during the continuation treatment in childhood ALL. In one study, the dose intensity of mercaptopurine was the most important pharmacologic factor influencing treatment outcome. Overzealous use of mercaptopurine is counterproductive, however, resulting in interruption of chemotherapy because of neutropenia and reduction of overall dose intensity. Mercaptopurine should be taken in the evening and should not be taken with milk or milk products. 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 function abnormalities are tolerable and reversible.

A few patients (1 in 300) have an inherited homozygous deficiency of thiopurine S-methyltransferase, the enzyme that catalyzes the S-methylation (inactivation) of mercaptopurine. Mercaptopurine should be reduced markedly (eg, tenfold reduction) in these patients to avoid potentially fatal hematologic toxicity. Approximately 10% of patients are heterozygous for the enzyme deficiency and have intermediate levels of thiopurine methyltransferase. This subgroup can be treated safely with only moderate reductions in mercaptopurine dosage and appears to have better clinical outcomes than patients with the homozygous wild-type phenotype. Importantly, patients with this enzyme deficiency are at risk for therapy-related leukemia. Whether dose reduction of mercaptopurine can reduce the risk of therapy-related leukemia in these patients is unknown. Although thioguanine is more potent than mercaptopurine, leads to higher concentrations of thioguanine nucleotides in cells and cytotoxic concentrations in cerebrospinal fluid, and produces superior antileukemic response, its prolonged use has been associated with profound thrombocytopenia,

an increased risk of death, and an unacceptable rate of hepatic veno-occlusive disease. Therefore, mercaptopurine remains the drug of choice for ALL. The Clinical Pharmacogenetics Implementation Consortium recently 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.

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 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. Thus, whether this pulse therapy is necessary in contemporary regimens featuring early intensification of therapy remains to be determined.

CNS-directed treatment

Prophylactic cranial irradiation, once a standard treatment, is being replaced by intrathecal and systemic chemotherapy to reduce radiation-associated late complications. Two early clinical trials tested the feasibility of complete omission of prophylactic cranial irradiation from treatment; although the cumulative risks of an isolated CNS relapse were relatively low (4% and 3%), the event-free survival rates were only 68.4% and 60.7%. In another study, prophylactic cranial irradiation appeared to improve outcome in T-cell ALL with leukocyte count $>100 \times 10^9/L$. Thus, most childhood study groups continue to rely on prophylactic cranial irradiation for up to 20% of patients. A radiation dose of 12 Gy appeared to provide adequate protection against CNS relapse, even in high-risk patients (eg, those with T-cell ALL and leukocyte counts $>100 \times 10^9/L$). Two recent studies, however, tested the feasibility of total omission of prophylactic cranial irradiation, even in patients with T-cell ALL, hyperleukocytosis, or overt CNS leukemia at diagnosis. In these two studies, the 5-year survival rates were 85.6% and 81%, and the cumulative risks of an isolated CNS relapse were only 2.7% and 2.6%, respectively. Importantly, all 11 patients with isolated CNS relapse in the first study remained in second remission for 0.4 to 5.5 years. These promising results suggest that prophylactic cranial irradiation can be omitted safely in all patients in the context of the effective intrathecal and systemic chemotherapy.

Systemic treatment, including high-dose methotrexate, intensive asparaginase, and dexamethasone, and optimal intrathecal therapy are important to control CNS leukemia. Triple intrathecal therapy with methotrexate, cytarabine,

and hydrocortisone is more effective than intrathecal methotrexate alone in preventing CNS relapse. A recent 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 event-free survival, special precaution should be taken to decrease the rate of traumatic lumbar puncture (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 this is 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 headaches.

Stem cell transplantation

The indications for hematopoietic stem cell transplantation (SCT) during first remission should be reviewed continuously as treatment improves and new agents become available. At this time, poor early response to remission induction treatment (eg, $\geq 1\%$ blasts after remission induction), with the exception of patients ages 1-6 years with favorable leukemic cell genetics, is the most frequent indication for transplantation. Except in some small studies, transplantation failed to improve outcome of infant patients with *MLL* rearrangement. Hypodiploid cases did not appear to benefit from transplantation, but the number of patients treated with this modality was very small. The use of imatinib has dramatically improved early treatment results in children with *BCR-ABL1* positive ALL, including those with poor early response to chemotherapy, raising the question of whether transplantation should be performed in first remission even in children with this subtype of ALL. In fact, because of this remarkable early result, many pediatric oncologists are not recommending transplantation for children with *BCR-ABL1* positive ALL while awaiting the long-term results of the study. An ongoing international study of *BCR-ABL1* positive ALL is testing whether the intensity of chemotherapy can be reduced and cranial irradiation can be limited only to patients with overt CNS leukemia at diagnosis by substitution of imatinib with a more potent second-generation inhibitor (dasatinib) that readily penetrates into the CNS. Whether transplantation would improve the poor outcomes in patients with early T-cell ALL remains to be determined.

Treatment for relapse in children

Although an isolated hematologic relapse (blood and marrow) remains most common, relapses may involve extramedullary sites, such as the testes, CNS, lymph nodes, skin, liver, spleen and other organs. Factors indicating an especially poor prognosis include relapse while on therapy or after a short initial remission, T-cell immunophenotype, and an isolated hematologic relapse. Prolonged second remissions (>3 years) can be achieved with chemotherapy in as many as half of patients with late relapses (ie, >6 months after cessation of initial maintenance chemotherapy) but in only $\sim 10\%$ of patients with early relapse. The presence of MRD after reinduction treatment also portends a very poor prognosis. In patients who experience hematologic relapse while on therapy or shortly thereafter and in patients with high levels of MRD after remission induction for relapse, allogeneic hematopoietic SCT is the treatment of choice.

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 for haploidentical transplantation. Among various therapeutic regimens tested in relapsed ALL, the combination of clofarabine, etoposide, and cyclophosphamide, and the addition of bortezomib, a protezomib inhibitor, to a standard four-drug induction appears to be promising and warrants additional studies. In one randomized trial, patients received mitoxantrone during induction had superior progression-free survival than those treated with idarubicin, a finding being validated in an ongoing international trial.

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 testicular relapses. Importantly, submicroscopic bone marrow involvement at a level of 10^{-4} or higher at the time of overt extramedullary relapse confers a very poor outcome. Hence, patients with extramedullary relapse and MRD in bone marrow require intensive treatment to prevent subsequent hematologic relapse. The efficacy of retrieval therapy in children with an isolated CNS relapse depends partly on duration of CR1 and partly on whether CNS irradiation was previously performed. The strategy of delaying cranial or craniospinal irradiation for 6-12 months to allow initial intensification of systemic chemotherapy has yielded long-term second event-free survival rates of 70%-80% in children with isolated CNS relapse. In one study, 12 months of intensive systemic chemotherapy and reduced-dose cranial

irradiation (18 Gy) resulted in an excellent 4-year event-free survival rate among children with B-cell ALL who had not received cranial irradiation during initial treatment and had an initial remission duration of >18 months. One-third of patients with early testicular relapse and two-thirds of patients with late testicular recurrence became long-term survivors after salvage chemotherapy and testicular irradiation. For patients with bilateral testicular relapse, local irradiation (22–26 Gy) usually is recommended, but the optimal dose of irradiation is unclear. In patients with unilateral testicular relapse, some leukemia therapists advocate unilateral orchiectomy with reduced irradiation (15 Gy) to the “uninvolved testicle,” but others would rely on intensive chemotherapy alone to spare the testicular function. Indeed, successful treatment in some patients with testicular relapse has been achieved without the use of any testicular irradiation.

Targeted therapies

The best example of targeted therapy is the use of the TKI imatinib in *BCR-ABL1* positive ALL. Second-generation TKIs (eg, dasatinib, nilotinib) that are more potent have been developed to partly address the problem of resistance to imatinib. Other novel agents include inhibitors of FLT3, JAK, farnesyltransferase, proteasome, DNA methylation, and histone deacetylase. Immunotherapeutic options are progressively emerging. Rituximab (anti-CD20), gemtuzumab ozogamicin (anti-CD33), alemtuzumab (anti-CD52), and epratuzumab (anti-CD22) already have been incorporated into some clinical trials. Recombinant immunotoxins (inotuzumab ozogamicin) and bispecific antibodies (blinatumomab) currently are being tested. Other promising drugs include nelarabine and the investigational agent forodesine, both for T-cell ALL.

Special subgroups of ALL in children

Patients with Down syndrome have a 10- to 20-fold higher relative risk for leukemia, and they constitute ~2% of pediatric ALL. They have the same age range as the general pediatric population with the exception of a lack of cases in the infant age-group. ALL patients with Down syndrome 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 have a high frequency of activating somatic *JAK2* mutations, affecting approximately 20% of the cases. A recent study showed that 55% of Down syndrome cases have *CRLF2* overexpression, frequently in association with activating *JAK* mutations. Although the outcome has improved with modern treatment, these patients still fared significantly worse than other

children with ALL, because of poor tolerance to chemotherapy, such as dexamethasone and methotrexate, and excessive treatment-related deaths.

Infant ALL accounts for 2%-3% of childhood ALL and is characterized by a high frequency of 11q23 chromosomal abnormalities and rearrangements of the *MLL* gene (70%-80%), a CD10-negative pro-B immunophenotype, hyperleukocytosis, and an inferior outcome. A clinical trial featuring intensive lymphoid- and myeloid-directed therapy resulted in improved outcome of infants with ALL, with a 4-year event-free survival rate of 47%.

Several studies have shown that adolescents and young adults (ages 15–39 years) treated on pediatric trials fared significantly better than the same age-groups treated on adult protocols. The superior outcome with pediatric regimens has been attributed to more effective treatment and to the better treatment adherence by patients, parents, and clinicians, although this remains an important topic of investigation. Several combined adult and pediatric consortia are using common regimens to treat children and young adults to understand the basis for this difference.

Treatment of B-lymphoblastic and T-cell ALL in adults

Tailoring treatment to assessed risk has resulted in improved outcomes in pediatric ALL. In adults with ALL, although risk stratification has been used with success in the treatment of Burkitt ALL and, more recently, *BCR-ABL1* positive ALL (described later), over the past two decades, the majority of patients with B- and T-cell ALL have been treated without specific consideration of biologic risk. Treatment for these adults, in general, has followed the same basic strategy of 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. Nevertheless, the use of regimens patterned after those used in childhood ALL has resulted in the achievement of remission in the majority (75%-90%) of adults with ALL, although cure rates are only ~30%-40% overall. The general treatment strategy for adults with ALL is described in the following sections. Because of the lower survival rates in adults with ALL treated with aggressive combination chemotherapy approaches, the use of allo-SCT in CR1 has been explored, 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 disease subset, as described in the following sections, for adolescents and young adults with ALL and those with Ph+ ALL.

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 achieving remission in many current multicenter studies. 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%. 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'Adulto* (GIMEMA) reported that, similar to childhood ALL, a good response to 1 week of pretreatment prednisone before chemotherapy, defined as a decrease in circulating blasts to 1,000/ μ L, was predictive of a longer CR duration and survival. An alternative treatment regimen known as Hyper-CVAD was developed at the M.D. Anderson Cancer Center and uses hyperfractionated cyclophosphamide (similar to the approach used for Burkitt lymphoma/leukemia), dexamethasone, vincristine, and doxorubicin without asparaginase during induction and high-dose cytarabine and methotrexate during consolidation. In their trial of 204 patients, 91% achieved CR with 3- and 5-year DFS rates of 50% and 38%, respectively.

Pioneered in the treatment of pediatric ALL, the contribution of asparaginase to response rates and duration of response in adults is not clear as there are no randomized studies supporting its use in adult patients. The toxicities of asparaginase in adults include pancreatitis, hepatotoxicity, and coagulopathy. An analysis of the Cancer and Leukemia Group B (CALGB) 8811 study showed a marginal benefit in DFS at 3 years for patients who received all prescribed doses of asparaginase (55% vs. 48%, with overlapping 95% confidence intervals). Eighty-five percent of the 197 patients in that trial achieved CR after induction. Ongoing trials by the German Multicenter ALL (GMALL) group of the long-acting asparaginase, PEG-asparaginase, 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/ μ L, patients receiving G-CSF had significantly shorter hospital stays, less time to neutrophil recovery, and fewer severe infections compared with patients who did not receive G-CSF. The previously discussed CALGB 9111 trial highlighted the benefit of using this drug in patients prone to difficulty with hematologic recovery, specifically older patients. The study observed a trend toward increased CR rates in patients 60 years of age 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 postremission 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 postremission treatment. The postremission 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 postremission 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 is 50%-75%, leading to many variations of postremission consolidation treatment in an attempt to eradicate MRD and improve DFS. Adult consolidation regimens have evolved from pediatric schedules that have been shown to be successful. Postremission therapy in ALL can include a wide range of drugs, including cytarabine, etoposide, teniposide, methotrexate, mercaptopurine, and thioguanine. In addition, the use of autologous SCT (auto-SCT) and allo-SCT 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 II study. The results showed that median remission duration improved to 29 months, whereas median survival extended to 36 months. Likewise, the M.D. Anderson group used extended consolidation within its Hyper-CVAD regimen by alternating cyclophosphamide, doxorubicin, vincristine, and dexamethasone (cycles 1, 3, 5, and 7) with high doses of methotrexate and cytarabine (cycles 2, 4, 6, and 8) in a single-arm trial. In this study of 204 patients treated between 1992 and 1998, median survival time was 35 months, and the 5-year survival rate was 39%. The Italian GIMEMA group conducted a study that included randomization of 388 patients to postremission intensification followed by

maintenance chemotherapy versus early maintenance therapy without intensification.

In summary, all of these regimens result in similar DFS rates of ~30%-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 >60 years old still have a dismal prognosis, with <10%-15% achieving long-term survival. Ongoing clinical trials are testing the incorporation of novel agents, including targeted monoclonal antibodies, such as rituximab for CD20 positive lymphoblasts and alemtuzumab for CD52 positive cases, as well as TKIs into standard regimens in an attempt to eradicate MRD and improve DFS.

CNS prophylaxis

Although <10% of adults with ALL will present with CNS involvement, CNS relapse will occur in 35%-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 always is performed in pediatric studies, but this typically is delayed in most adult ALL regimens. Unless a patient has CNS symptoms, the CALGB regimens perform an initial lumbar puncture at the start of consolidation (postremission) chemotherapy. 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.

Initially, CNS-directed therapy included the use of intrathecal methotrexate and 24 Gy of cranial radiation in the pediatric population. This strategy was incorporated into an early adult trial that compared CNS prophylaxis with no CNS treatment, resulting in an improved CNS relapse rate of 19% versus 42% at 24 months. Although in children it is known that combination treatment can result in toxicities that include seizures, early dementia, cognitive dysfunction, and slow growth, the long-term effects on adults are less clear. It is known that combined radiation and intrathecal chemotherapy in adults can cause substantial acute toxicities that may delay postremission consolidation treatment. An alternative strategy that combines intrathecal chemotherapy without radiation has been investigated. This treatment

regimen includes so-called *triple therapy* that uses intrathecal methotrexate, cytarabine, and corticosteroids without radiation.

CNS relapse rates as low as 5% have been achieved without radiation by using combination intrathecal treatment in conjunction with high-dose systemic treatment that can penetrate the cerebrospinal fluid. The German GMALL investigators have reported higher CNS relapse rates of 9% versus 5% when CNS-directed radiation was postponed.

Therefore, although CNS-directed prophylactic therapy is required in ALL treatment, there is no single modality or combination that has been proven to be superior. Of note, the pediatric groups generally recommend cranial irradiation as part of CNS prophylaxis for high-risk T-cell ALL, as do the German study groups.

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 up to 3 years after initial diagnosis. Commonly used components of maintenance therapy include daily mercaptopurine and oral weekly methotrexate, supplemented by monthly pulses of vincristine, corticosteroids, and periodic intrathecal methotrexate.

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, maintenance regimens mimicking those used in pediatric protocols routinely are incorporated into the treatment regimens of adult B- and T-cell ALL.

Risk-directed treatment of adult ALL

The focus of current treatment studies for adults with ALL is to begin to adapt treatment according to biologic risk. Similar to the progress that has been made in Burkitt lymphoma/leukemia when a specific therapeutic approach is applied based on the underlying biology of the disease, targeted treatment of Ph+ ALL that incorporates the TKIs imatinib or dasatinib into frontline therapy and the application of a pediatric-inspired regimen for older adolescents and young adults may be changing the treatment paradigm for adults with ALL, resulting in improvements in survival. The role of allo-SCT in first remission based on risk group also is reviewed.

BCR-ABL1 positive ALL

Treatment and outcome of patients with *BCR-ABL1* positive ALL has changed dramatically during the past decade with the addition of imatinib, a targeted ABL TKI, to frontline therapy. Previously, the standard approach for adult patients in whom the presence of the Ph chromosome portended a very poor prognosis with standard therapy alone (median survival of 1 year) was to recommend, whenever possible, allo-SCT in first remission. Allo-SCT in first remission resulted in improved DFS rates ranging from 30% to 65% in the pre-imatinib era. Recent studies have demonstrated that the addition of the TKI, imatinib or dasatinib, into frontline chemotherapy is feasible, does not add to systemic toxicities, and significantly increases remission rates. Several studies have reported CR rates >90% for these high-risk patients. Results from these studies also suggest that the addition of TKIs to standard therapy can rapidly reduce MRD. Because it has been demonstrated that patients with *BCR-ABL1* positive ALL have improved DFS when allo-SCT is performed without evidence of MRD, the addition of TKIs before or after allogeneic transplantation appears to be improving OS in this high-risk group. When imatinib is added to frontline therapy, followed by allo-SCT (and sometimes followed by post-SCT imatinib), DFS rates of 60%-75% have been reported in these studies. For patients >60 years old with B-cell ALL for whom the incidence of the *BCR-ABL1* positive disease approaches 50%, the addition of TKIs to standard regimens appears to be improving remission duration, even without the addition of allo-SCT. Longer follow-up will be needed, however, to confirm these promising results. The emergence of TKI resistance remains the largest obstacle to long-term survival. Thus, new trials are under way that incorporate the third-generation TKI ponatinib, which has an advantage over current TKIs as it maintains activity in cases with a variety of ABL kinase domain mutations that result in TKI resistance, including the T315I.

In summary, the addition of molecularly targeted therapy in ALL has created a new paradigm for improving outcome for these high-risk patients. At the present time, incorporation of a targeted TKI into frontline therapy followed by allo-SCT in first remission for eligible patients appears to be the standard of care. For patients who are not suitable candidates for transplantation, current studies are evaluating the benefit of prolonged TKI therapy in combination with more traditional CNS prophylaxis and maintenance chemotherapy.

Adolescents and young adults

Increasing age is one of the most important poor prognostic factors of outcome in newly diagnosed patients with ALL. The 5-year DFS is approximately 80% for children and 40%

for adults with ALL. These divergent outcome results can be explained, in part, by the much higher incidence of poor-risk cytogenetics (eg, Ph+) and the lower incidence of favorable-risk molecular genetics (eg, *RUNX-ETV1*) in older adults with ALL. In addition, older patients with ALL have a higher incidence of associated comorbid conditions with poorer baseline performance status, which frequently preclude the use of intensive chemotherapy regimens and enrollment onto clinical trials. Recent retrospective data suggest that younger patients between the ages of 16 and 21 years fare better when treated according to current intensive pediatric regimens rather than with conventional adult ALL treatment regimens. Despite slight differences in treatment approaches across the different cooperative groups, all of the retrospective studies have demonstrated significantly better outcome for the patients when treated on pediatric studies, where survival has been reported to be in the range of 60% to 65%. In contrast, when the same age-group is treated on adult cooperative group ALL treatment trials, survival has been only 30%-40%.

From these retrospective studies, it appears that the major differences in treatment between the adult and pediatric regimens are the more intensive use of the nonmyelosuppressive agents (glucocorticoids, asparaginase, and vincristine), earlier and more intensive CNS-directed therapy, and more prolonged maintenance therapy used in the pediatric regimens. In addition to the obvious treatment differences between adult and pediatric trials, there has been much debate about potential differences in adherence to protocol therapy among pediatric and adult medical hematologists and the patients that they treat.

Several new prospective European and American studies that apply the pediatric approach to younger adults recently have been published and confirm promising outcomes for patients ages 16-30 years old, with reported DFS rates of 60%-65%. The Dana-Farber consortium has presented preliminary results applying this pediatric approach to adults up to the age of 50 years with an event-free survival rate of 63% with short follow-up. The U.S. Intergroup is performing a large phase II trial (C-10403) for younger adults up to age 40 years that is testing the successful approach used by the Children's Oncology Group for treatment of high-risk adolescents with ALL. Older adults >45 years of age, however, may not benefit from this approach, in part, because of their inability to tolerate the intensive asparaginase, glucocorticoid, and vincristine dosing upon which these regimens are based.

Allo-SCT in adult ALL in CR1

The efficacy of allo-SCT in ALL was first reported in 1973, and the graft-versus-leukemia effect was described as early

as 1979. The role of allo-SCT has been established in patients with well-known risk factors, such as t(9;22) and t(4;11) cytogenetics, and may represent the optimal approach to curing these patients. Determining whether other patients also may benefit from allo-SCT in CR1 has been an area of intense study. Results from recent trials suggest that specific disease subsets may benefit from an allo-SCT in CR1.

In an earlier trial from the Netherlands, 54 patients (ages 15–51 years) with ALL and 15 patients with lymphoblastic lymphoma were treated with induction and consolidation chemotherapy. Thirty patients had an HLA-matched sibling, and 22 of those patients were scheduled to undergo allo-SCT. The DFS of these patients was $58\% \pm 11\%$ at 5 years, a result not significantly different from the outcomes of the other patients in the study who did not receive transplantation as part of their regimens.

The French LALA-87 trial attempted to evaluate the best postremission strategy in ALL, comparing consolidation chemotherapy versus auto-SCT versus allo-SCT. The results of this trial analyzing 572 patients with 10 years of follow-up data showed that survival was 46% for patients who received an allo-SCT versus 31% for patients who received chemotherapy ($P = 0.04$). When broken into high-risk and standard-risk groups (with high risk including Ph+ status, age >35 years, WBC $>30 \times 10^6/\mu\text{L}$, and time to CR >4 weeks), OS at 10 years was 44% in the allo-SCT group versus 11% for the chemotherapy group ($P = 0.009$). In the standard-risk group, survival rates in the allo-SCT group (49%) and the chemotherapy group (39%) were similar ($P = 0.6$). Thus, this study demonstrated a survival benefit for allo-SCT in CR1, but was limited only for the high-risk patients.

Similarly, the LALA-94 trial reevaluated the benefit of allogeneic transplantation in high-risk patients. In this study, 922 adult patients were divided into the following four risk groups: (i) standard-risk ALL, (ii) high-risk ALL, (iii) Ph+ ALL, and (iv) ALL with CNS involvement. Patients in all but the standard-risk group were assigned to receive allo-SCT if they had an HLA-matched sibling. Patients in groups 3 and 4 were assigned to auto-SCT if no family donor was available, whereas patients in group 2 were randomized to either auto-SCT or further chemotherapy. The results of this intent-to-treat analysis showed that patients with high-risk ALL and patients with CNS involvement had a better outcome if a donor was available for transplantation. Among high-risk patients, those allocated to the allo-SCT arm had a better median DFS of 20.8 months compared with a median DFS of 15.2 months in the auto-SCT arm and a median DFS of only 11 months in the chemotherapy arm ($P = 0.007$). These results confirm the findings of the LALA-87 trial showing benefit of allo-SCT in high-risk patients if a sibling donor is available.

The Medical Research Council United Kingdom ALL (MRC UKALL) 12/Eastern Cooperative Oncology Group (ECOG) 2993 study is the largest prospective, randomized trial comparing allo-SCT with chemotherapy as a postremission treatment strategy. In this study, 1,913 patients ages 15–59 years were enrolled between 1993 and 2006, with the upper age limit extended to 64 years in 2003. The study schema allocated all patients <50 years (later amended to 55 years) having an HLA-matched sibling to receive a transplantation. All Ph+ patients were assigned to transplantation, using a matched-unrelated donor if necessary. Younger patients without a family member donor and patients >50 years (or 55 years old later in the study) were randomized to either auto-SCT or further chemotherapy for consolidation treatment. High-risk patients throughout the study period were defined by the following factors: (i) age >35 years, (ii) WBC count $>30,000/\mu\text{L}$ in B-lineage disease or $>100,000/\mu\text{L}$ in T-lineage disease, and (iii) Ph+ status. The median follow-up time was 4 years, 11 months (range, 1 month to 13 years, 11 months). Ph chromosome-negative patients with a donor had a 5-year OS rate of 53% versus 45% for patients without a donor ($P = 0.01$). In stark contrast to the two LALA trials discussed previously, standard-risk patients were the only group to benefit from transplantation, with 5-year OS rates of 62% versus 52% ($P = 0.02$) in patients who had a donor versus those who did not, respectively. The benefit from transplantation in high-risk patients was not statistically significant ($P = 0.2$), with OS rates of 41% and 35% in the donor group and no-donor group, respectively. The trial also showed that in all groups, auto-SCT offered no more benefit than chemotherapy alone. As opposed to the LALA trials, the joint MRC UKALL/ECOG trial showed that transplantation was most beneficial to standard-risk patients, as defined, rather than high-risk patients. Thus, allo-SCT in CR1 was not significantly better for patients >35 years old (high risk) because of unacceptably high transplantation-related mortality.

Auto-SCT has been studied as a treatment option for patients who do not have an HLA-matched sibling donor. In a review of the French LALA-85, -87, and -94 trials, investigators studied 175 patients who received auto-SCT and 174 patients who were treated with chemotherapy. Their results showed that receiving auto-SCT was associated with a lower incidence of relapse compared with treatment with chemotherapy (66% vs. 78% at 10 years, respectively; $P = 0.05$). DFS and OS, however, were not significantly different between the groups. Similarly, Yanada, Matsuo, et al. (2006) performed a meta-analysis of seven trials conducted by Japanese and European cooperative groups that included 1,274 patients and reported no benefit when auto-SCT was compared with chemotherapy in patients who lacked an HLA-matched sibling donor.

Relapsed disease

Although the majority of adult ALL patients reach CR, most eventually will relapse and subsequently be much less responsive to salvage therapy. First relapse typically occurs within the first 2 years after induction, and remissions lasting longer than 18 months are associated with improved response to salvage regimens. CR rates for salvage regimens range from 31% to 78%, and survival for these patients remains poor. The more effective salvage regimens are multidrug regimens and usually contain intermediate- to high-dose cytarabine. Clofarabine, a novel purine nucleoside analog, is approved for relapsed ALL in children. Its use in adults as a single agent or in combination is less well studied. Recently, liposomal vincristine (Marqibo) was approved for adult patients with second relapse of their disease.

In patients with relapsed or refractory T-cell 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. Despite this difficult patient population, nelarabine allowed patients to proceed to transplantation and achieve increased survival. On the basis of the significant activity in relapsed disease, several studies have incorporated nelarabine into frontline therapies in hopes of improving the outcome of patients with newly diagnosed T-cell ALL.

Allo-SCT beyond CR1

To evaluate the role of SCT in relapsed disease, the large MRC UKALL 12/ECOG 2993 trial evaluated the outcome of 609 relapsed patients treated with chemotherapy, auto-SCT, or allo-SCT. The 5-year OS rates for the chemotherapy, auto-SCT, matched unrelated-donor SCT, and sibling SCT arms were 4%, 15%, 16%, and 23%, respectively, with a significant survival difference between the chemotherapy and transplantation groups. The LALA-94 trial observed similar results in relapsed patients with active disease or in second CR (CR2), with SCT producing improved DFS and OS with a 5-year OS rate of 25%. In these trials, initial postremission therapy and risk stratification group did not affect relapse rates; however, achieving CR2 before SCT did improve outcomes. These studies, as well as previous studies, show that allo-SCT is the only potentially curative therapy in relapsed or refractory ALL. Available data from the Center for International Blood and Marrow Transplant Research (CIBMTR) show that patients receiving transplantation with an HLA-

identical sibling donor for ALL in CR2 have a ~35%-40% chance of long-term DFS, whereas patients receiving transplantation with disease not in remission have a DFS of only 10%-20%.

Novel therapies

Several new agents are in clinical trials for patients with relapsed disease, some of which are now being incorporated into frontline therapies. These include: (i) monoclonal antibodies, such as rituximab, alemtuzumab, epratuzumab, novel immunoconjugated antibodies directed against CD22 (inotuzumab ozogamicin), or the bispecific CD19:CD3 antibody (blinatumomab); (ii) drugs that target aberrant NOTCH1 expression resulting from activating mutations in NOTCH1, which have been reported in up to 60% of both adults and children with T-cell ALL; and (iii) the newer generation of ABL (or multitargeted) TKIs, including nilotinib, dasatinib, and ponatinib. Exploration of allo-SCT using reduced-intensity conditioning regimens is another novel approach to the treatment of high-risk patients that might result in a potent antileukemia effect while minimizing the unacceptably high treatment-related mortality. Of note, patients >60 years old with ALL, in whom survival remains dismal at <10%, are excellent candidates for trials that incorporate some of these novel approaches into frontline treatment.

Key points

- The prognosis of adolescent and adult ALL has improved, but cure rates remain inferior compared with children with ALL. The majority of adults (75%-90%) will achieve remission, but OS at 5 years remains at 30%-40%.
- Cytogenetic abnormalities provide important prognostic information and are used for risk-adapted therapies. MRD detection using sensitive methods identifies patients at high risk of relapse.
- Therapy for B-lymphoblastic and T-cell ALL consists of multiagent induction, consolidation-intensification, CNS prophylaxis, and maintenance phases. These regimens are typically modeled after the successful regimens used by pediatricians; however, alternative regimens have been used with equivalent results.
- Treatment outcomes for older adolescents and young adults appear to be improving with the use of pediatric regimens that focus on dose-intensive glucocorticoids, vincristine, and asparaginase.
- Allo-SCT in CR1 has been demonstrated to improve survival of specific subsets of patients in several studies; however, the optimal selection of patients for this approach remains controversial. Allo-SCT is recommended as the only potentially curable strategy for eligible patients in CR2.

Key points (continued)

- The prognosis for patients >60 years of age remains poor, with a DFS rate of <10%-20%. This primarily reflects B-lineage Philadelphia chromosome-negative as T-lineage is quite rare in older adult patients. Therefore, older adults with ALL are excellent candidates for novel therapeutic approaches.
- Treatment of Ph+ ALL now includes the addition of a targeted TKI to frontline therapy, which has improved remission rates and prolonged DFS. Currently, eligible adult patients are still recommended to receive an allo-SCT in CR1. Studies are under way to determine the utility of posttransplantation TKI therapy and to evaluate the effect of prolonged TKI therapy in combination with lower dose chemotherapy for older adults with Ph+ ALL who are not transplantation candidates. The DFS rates approach 30%-40% in older patients with Philadelphia chromosome-positive ALL who receive TKI-based therapy and undergo stem cell transplantation in first CR.
- Therapy for Burkitt ALL in adults is modeled after successful pediatric regimens using intensive short-course cyclic therapy and has resulted in significant improvements in outcome for adults, with OS rates of 50%-60%. Recent studies incorporating rituximab, a monoclonal antibody directed against CD20, have resulted in even more promising survival rates of >70%.

71 (72%) of 99 children with newly diagnosed T-cell lymphoblastic lymphoma, a proportion that is much higher than that previously established by morphologic examination. The levels of involvement ranged from 0.01% to 31.6%. Interestingly, lymphoma cells were as prevalent in peripheral blood as they were in bone marrow, suggesting that examination of blood samples can be used for disease staging and monitoring in these patients. Moreover, high levels of disease dissemination ($\geq 1\%$) were significantly associated with a poorer event-free survival.

The treatment strategy for lymphoblastic lymphoma is similar to that used for T-cell ALL. Intensive multiagent systemic chemotherapy regimens incorporating CNS-directed therapy have resulted in event-free survival rates of 75%-90% in children and 40%-80% in adults. The Berlin-Frankfurt-Münster 95 trial showed that prophylactic cranial irradiation could be omitted from treatment in patients with stage III or IV disease and without CNS involvement at diagnosis. In a single-institution pilot study, a 5-year event-free survival rate of 83% and a 5-year OS rate of 90% were achieved without the use of cranial irradiation in all patients, regardless of the CNS status at diagnosis, a result similar to that for childhood ALL.

Lymphoblastic lymphoma

Lymphoblastic lymphoma represents ~2%-30% of adult and pediatric non-Hodgkin lymphomas, respectively. The peak incidence is in the second decade of life, with a smaller peak in adults >40 years of age. Males are affected twice as often as females. The vast majority of patients have advanced-stage disease with a precursor T-cell immunophenotype. The immunophenotype of T-cell lymphoblastic lymphoma overlaps with that of T-cell ALL. The clinical distinction between these two entities is determined arbitrarily by the degree of bone marrow involvement. Patients with $\geq 25\%$ bone marrow replacement by lymphoblasts are considered to have T-cell ALL, whereas patients with a lesser degree of replacement or no detectable abnormal lymphoblasts in the marrow are classified as having T-cell lymphoma. In fact, lymphoma cells can be detected in the bone marrow in <20% of patients with T-cell lymphoma using conventional morphologic examination of bilateral bone marrow aspirates and biopsies. Distinguishing lymphoma cells from normal activated lymphocytes and lymphoid progenitors (hematogones) by morphology alone can be difficult, and hence, the true extent of disease dissemination at diagnosis in T-cell lymphoma was uncertain in the past. Using a flow cytometric method that allows for the detection of 1 lymphoma cell among 10,000 normal cells, it recently has been reported that marrow involvement was present in bone marrow samples of

Late complications of therapy

Emphasis on the intensive use of methotrexate and glucocorticoids has led to an increased frequency of neurotoxicity and osteonecrosis, underscoring the need for judicious use of even seemingly benign agents. Many long-term survivors of childhood ALL, especially those who received high cumulative doses of glucocorticoid, methotrexate, or cranial irradiation, have developed severe osteoporosis. Such development highlights the need for early identification of bone lesions and therapeutic intervention to prevent fractures. Treatment with anthracyclines can produce severe cardiomyopathy, especially when they are given in high cumulative and peak doses to young girls. Cardiac abnormalities are persistent and progressive years after anthracycline therapy. In one study, dextrazoxane prevented or reduced anthracycline-induced cardiotoxicity without interfering with antileukemic activity. In current clinical trials, only limited doses of anthracyclines are used, even for high-risk cases, to decrease the risk of subsequent cardiomyopathy.

Cranial irradiation has been implicated as the cause of numerous late sequelae in children, including second cancer, 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 clearly are linked to cranial irradiation, they also can be caused by systemic and intrathecal therapy. Knowledge of potential treatment sequelae to modify treatment strategy and of appropriate screening measures to permit early detection of complications should greatly improve the quality of life of survivors of ALL.

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Hodgkin lymphoma

Kristie A. Blum and Ann S. LaCasce

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CHAPTER **20**

Hodgkin lymphoma

Kristie A. Blum and Ann S. LaCasce

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Introduction

Hodgkin lymphoma (HL) represents ~10% of all cases of malignant lymphoma. The disease typically affects young adults and presents with painless lymphadenopathy involving the neck and chest. Systemic symptoms of fevers, night sweats, and unexplained weight loss are common. The vast majority of patients with HL are cured with combination chemotherapy with or without radiation. Given the long-term survival of patients with Hodgkin lymphoma, current efforts are focused on reducing late, treatment-related toxicities.

Epidemiology

Approximately 9,000 patients per year in the United States are diagnosed with HL. HL is divided into two distinct entities, classical Hodgkin lymphoma (cHL) (95% of cases) and nodular lymphocyte-predominant Hodgkin lymphoma (*nLPHL*). The median age at diagnosis is 24, although the disease has a bimodal age distribution with one peak in the early 20s and the second in the mid-60s. There is a slight male predominance.

Pathology

The malignant cell in HL is the Hodgkin Reed Sternberg (HRS) cell, a large, bi-lobed cell with two or more nuclei with eosinophilic nucleoli. There are several morphologic variants

of HRS cells, including the: (i) mononuclear variant “Hodgkin cell”; (ii) “Lacunar cells,” which have multilobulated nuclei with small nucleoli and, during tissue fixation, the ample cytoplasm retracts leaving a lacunae or space around the nucleus; (iii) “mummified cells” that have condensed cytoplasm with pyknotic, red nuclei; and (iv) the “lymphocytic and histiocytic” (L&H) or “popcorn cells,” which are seen in *nLPHL* and have lobulated, vesicular nuclei with multiple small nucleoli that are located peripherally. For many years, the cell of origin of the HRS cell was unknown. Using single-cell microdissection techniques, HRS cells were isolated from histologic sections of HL and analysis by PCR identified clonal immunoglobulin genes with evidence of somatic mutation, thus proving that HRS cells were derived from germinal center cells. HRS cells account for the minority of cells, from as little as 1%, in HL, and are surrounded by a background of mixed inflammatory cells, which varies according to the histologic subtype and includes B- and T-cells, plasma cells, eosinophils, neutrophils, macrophages, and fibroblasts.

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. Within classical cHL, there are four histologic subtypes: nodular sclerosis (*nS*), mixed cellularity, lymphocyte rich (LR), and lymphocyte depleted (LD). In cHL, the HRS express CD30 in nearly all cases and CD15 in ~85%. CD20 is positive in ~20% of cases, but other B- and T-cells markers, including CD45, typically are absent. The B-cell transcription factors, OCT-2 and BOB1 typically are decreased. BSAP (PAX-5) also is expressed in HRS cells and its presence can be helpful in distinguishing the disease from anaplastic large-cell lymphoma,

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Off-label drug use: Dr. Blum: Rituximab for the treatment of lymphocyte predominant Hodgkins. Dr. LaCasce: Rituximab for the treatment of lymphocyte predominant Hodgkins.

which also expresses CD30 and may have large, atypical cells. Epstein-Barr virus (EBV), as evidenced by LMP-1 or EBV small nuclear transcripts (EBER), is found in a subset of cHL, in more than half of cases of mixed cellularity (75%), and in nearly all cases of LD HL.

Nodular sclerosis (*nS*) is composed of nodular areas with fibrous bands. The HRS cells may be rare and are often of the lacunar cell variant, but they also may be found in sheets (the syncytial variant). Neutrophils, eosinophils, and macrophages make up the inflammatory background. 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 appearance is most commonly diffuse. LR typically appears nodular but also can be diffuse. Typical HRS are present, but mononuclear cells and cells that are similar to those seen in *nLPHL* (L+H cells) are seen. The background is composed predominately of small lymphocytes. The least common subtype, LD, has a diffuse histologic appearance with a large number of HRS cells, which may appear to be atypical, in a background of fibrosis and necrosis with few inflammatory cells.

Pathogenesis

Although HRS cells are derived from germinal center B-cells in the vast majority of cases, 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. As such, the B-cell transcription factors OCT-2, BOB-1, and PU.1 are not expressed and signaling through the B-cell receptor is downregulated. The nuclear factor kappa B (*nF-kB*) and Janus kinase–signal transducer and activation of transcription signaling (JAK–STAT₃ pathways have been implicated as a key components of the growth and survival of HRS cells.

The microenvironment in cHL is critical to the survival of the HRS cell. The HRS secretes a variety of cytokines and chemokines, including tumor necrosis factor alpha (TNF α), interleukin-4 (IL-4), IL-5, IL-6, chemokine ligand-5 (CCL5), CCL17, CCL22, and fibroblast growth factor (FGF), that influence the surrounding inflammatory background. In addition, the surrounding cells, including T- and B-cells, macrophages, neutrophils, plasma cells, eosinophils, and macrophages also express multiple cytokines and chemokines, including IFN (interferon) gamma, IL-2, IL-10, and IL-13, which provide signaling that supports the growth and survival of the HRS cells.

Risk factors

In 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, and has been

postulated to be related to a delayed exposure to common childhood illnesses or environmental factor. A diagnosis of infectious mononucleosis also confers an increased risk for the subsequent development of cHL. In the developing world and areas of lower socioeconomic status, however, 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, either from HIV infection, in the setting of solid organ or hematopoietic stem cell transplant, or who are treated with immunosuppressive medications for autoimmune or inflammatory disease, are at higher risk for the development of cHL, typically associated with EBV. The risk of HIV-associated cHL has risen in the era of highly active antiretrovirals for unclear reasons. In addition, the risk of cHL is increased in patients with autoimmune disease, including rheumatoid arthritis, lupus, and sarcoidosis.

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 is increased approximately 100-fold.

Key points

- 9,000 new cases of HL are diagnosed per year in the United States.
- cHL represents 95% of cases with the remainder being *nLPHL*.
- There are four histologic subtypes of cHL: NS, mixed cellularity, LR, and LD.

Clinical presentation

Patients with cHL typically present with nontender lymphadenopathy, with the neck being the most commonly involved site of disease. B symptoms, defined as fevers >100.4, drenching night sweats, and weight loss of >10% of body weight in the preceding 6 months are common in patients with advanced stage disease but are present in <20% of patients with early stage disease. Pruritis, which may be intense and typically is not associated with a rash, although patients may develop secondary excoriations, is seen in 10%-15% of patients. Although it occurs rarely, patients may experience intense pain in the sites of disease upon alcohol ingestion.

The clinical presentation also varies according to the histologic subtype of HL. NS, 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 common and patients may present with respiratory symptoms. Mixed cellularity is the second-most-common subtype in the industrial world, representing 20% of cHL. The median age of presentation is 38, and males are affected more commonly. Patients typically present with peripheral lymphadenopathy. The mediastinum is less commonly

involved. Splenic involvement occurs in 30% and bone marrow occurs in 10%. 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 commonly is seen, and advanced stage disease and systemic symptoms are common.

Staging and workup

The Ann Arbor staging system has been employed in HL for more than 25 years and currently is used with the Costswolds modification (Table 20-1). X is assigned to bulky disease, defined as any mass >10 cm in transverse dimension or to an intrathoracic mass occupying greater than one-third of the maximal intrathoracic diameter, as measured at T5/6. The S designation refers to splenic involvement by disease.

Given that nearly all patients, including those with early stage disease, receive chemotherapy that treats microscopic disease, the use of staging laparotomy is no longer employed. In addition, modern imaging techniques, including computed tomography (CT) and functional imaging accurately identify splenic involvement by disease. Patients are staged with CT scans and positron emission tomography (PET), as HL is strongly fluorodeoxyglucose (FDG) avid. Recent studies have demonstrated a very high sensitivity for bony involvement. In addition, given that HL typically involves nodal stations in a contiguous manner, patients with asymptomatic disease above the diaphragm are exceedingly unlikely to have occult marrow involvement, likely obviating the need for marrow sampling in this subset of patients.

Patients should be evaluated with 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. Lactic 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.

Key points

- Patients with cHL typically present with painless lymphadenopathy in the neck and chest with or without B symptoms.
- Staging evaluation is performed with CT and PET scans with bone marrow biopsy under selective circumstances.
- Baseline evaluation includes an assessment of left-ventricular cardiac function and pulmonary function tests.

Frontline therapy for early stage HL

Clinical case

A 24-year-old woman presents with a persistent dry cough and diffuse pruritis without a rash for 2 months duration. On exam, she looks well and has no peripheral lymphadenopathy or splenomegaly. Chest x-ray reveals a widened mediastinum and subsequent chest CT is notable for a large 10.5 × 8 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. 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 shows mediastinal uptake less than blood pool, and she completes four cycles of chemotherapy followed by involved-field radiotherapy (IFRT) to 30 Gy.

Table 20-1 Staging of HL.

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, night sweats, or unexplained weight loss exceeding 10 percent 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 the level of T5/6 interspace or >10 cm maximum dimension of a nodal mass.

No subscripts are used in the absence of bulk.

Before the 1980s, patients with HL underwent laparotomy to fully stage the extent of disease. Patients without splenic involvement and disease confined to the neck and chest were treated with extended-field radiotherapy (EFRT), consisting of a mantle field and the para-aortic region. Approximately 95% of patients achieved a complete remission (CR), with 75% of patients remaining disease free in the long term. With the introduction of chemotherapy, multiple clinical trials subsequently demonstrated the superiority of chemotherapy plus radiation compared with radiation alone in terms of progression-free survival (PFS) and overall survival (OS).

Overall, the prognosis of early stage cHL using currently available therapies is excellent, with >85% of patients being cured of disease with initial therapy, and 95% of patients are alive at 5 years. Currently, the majority of patients with early stage cHL are treated with combined modality therapy given its superior freedom from treatment failure (FFT). Chemotherapy alone is associated with a higher risk of relapse but has significantly less long-term toxicity compared with combined modality treatment, and the OS is likely not different given the availability of effective second-line therapies.

Current approaches employ ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) over MOPP (mechlorethamine, vincristine, procarbazine, and prednisone), chemotherapy in terms of efficacy and toxicity, including risk of myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) and infertility based on a randomized study comparing MOPP, MOPP/ABVD, and ABVD (see full discussion in the section on frontline therapy for advanced-stage disease). The Stanford V regimen, which includes mechlorethamine, doxorubicin, vinblastine, prednisone, vincristine, bleomycin, and etoposide is an alternative regimen used in early stage cHL.

Radiation

Over time, the extent and dose of radiotherapy 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, EFRT also known as subtotal nodal radiotherapy (STNRT), includes both the involved lymph nodes and the grossly normal adjacent lymph nodes. Typical extended fields are the mantle field and the inverted Y field. IFRT, which encompasses only the clinically involved lymph nodes, replaced EFRT, in part based on two randomized studies. In the European Organization for Research and Treatment of Cancer (EORTC) H8 study, more than 1,500 patients were stratified according to risk (see detailed discussion in the following section). Chemotherapy consisted of MOPP/ABV. Favorable patients received three cycles of chemotherapy plus IFRT versus STNRT. Unfavorable risk patients received

four or six cycles of chemotherapy with IRFT versus four cycles of chemotherapy with STNRT. There was no difference in 5 year or 10 year event-free survival (EFS) or OS. Bonadonna et al. (2004) randomized patients to receive either STNRT versus IFRT following four cycles of ABVD chemotherapy. The freedom from progression (FFP) and OS at 12 years were equivalent in both arms at 94% and 96% respectively. Current studies are under way to evaluate the use of involved-node radiotherapy, in which the prechemotherapy lymph nodes plus an additional margin of ≤5 cm of surrounding, radiographically unininvolved tissue is treated.

In terms of the dose of the radiotherapy employed, most studies have used 20–40 Gy administered in 1.8–2 Gy fractions. The German Hodgkin Study Group (GHSG) analyzed two studies in which EFRT at 20 Gy, 30 Gy, or 40 Gy was administered following COPP/ABVD chemotherapy and demonstrated no difference in OS. Current studies typically employ 20–30 Gy of IFRT for nonbulky disease and 30–36 Gy of IRFT in the presence of bulk. Although the late toxicity of more limited radiotherapy fields and a lower dose of radiation likely will be reduced compared with that of larger fields, long-term follow-up will be required to confirm this hypothesis.

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 20-2). The GHSG scale includes five risk factors, including bulky mediastinal disease as defined by more than one-third of the maximal intrathoracic cavity, ESR of ≥30 in the presence of B symptoms or ≥50 without B symptoms, extranodal extension of disease, and three or more lymph node sites of involvement. The EORTC scale includes age ≥50 years, bulky mediastinal disease, ESR of ≥30 in the presence of B symptoms or ≥50 without B symptoms,

Table 20-2 Risk factors in early stage Hodgkin lymphoma.

EORTC	Age <50
	No LMA (less than one-third max intrathoracic diameter) ESR <50 without B sx ESR <30 with B sx <4 lymph node groups
GHSG	No LMA (less than one-third max intrathoracic diameter) ESR <50 without B sx ESR <30 with B sx No extranodal extension <3 lymph node groups

EORTC = European Organization for Research and Treatment of Cancer; ESR = Erythrocyte sedimentation rate; GHSG = German Hodgkin Study Group; LMA = large mediastinal mass.

and four or more nodal sites of involvement. In Canadian and some U.S. cooperative group studies, 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.

Favorable disease

In the EORTC H8F study, patients were randomized to three cycles of MOPP/ABV ($n = 270$) plus IFRT versus STNRT alone ($n = 272$). Both the 5-year EFS of 98% versus 74% ($p < .001$) and the 10-year overall 97% versus 92% ($P = .001$) favored the combined modality arms. The GHSG subsequently examined the role of fewer cycles of chemotherapy and lower dose radiation in the HD10 study: 1,370 patients without risk factors (fewer than three nodal sites, nonbulky disease without extranodal extension, ESR <30 without B symptoms or ESR <50 with B symptoms) were randomized in a two-by-two design to four versus two cycles of ABVD and 30 Gy versus 20 Gy of IFRT. With a median follow-up of 7.5 years, there was no difference in FFTF or OS at ~91%-93% and 97% at 5 years and 86%-90% and 94% at 8 years, respectively. Toxicity was comparable between all the arms. Overall, there have been 55 (4.6%) secondary malignancies, including 38 solid tumors, 15 cases of non-HL, and two cases of AML.

In the Canadian Eastern Cooperative Oncology Group (ECOG) H6 study, the use of chemotherapy alone was compared with STNRT with or without chemotherapy depending on risk factors. In the subset of patients with favorable disease—defined as age <40 , less than four lymph node sites involved, and absence of mixed cellularity or LD histology, ESR <50 —patients in the experimental arm received ABVD for four to six cycles ($n = 59$) depending on response. Patients who achieved CR following two cycles of ABVD received a total of four cycles, whereas all others received six cycles. The control arm consisted of STNRT ($n = 64$). The study was closed early after the EORTC study demonstrated the superiority of combined modality therapy using IFRT compared with STNRT. At a median follow-up of 11.3 years, there was no difference in the freedom from progressive disease at 89% and 87%, respectively, or OS at 98% in both arms. The outcome of patients who achieved CR after two cycles of ABVD was extremely favorable with freedom from disease progression and OS of 94% and 98%, respectively.

Unfavorable disease

In patients with unfavorable risk disease, The EORTC H8U study randomized 996 patients to three arms: four cycles of MOPP/ABV plus IFRT versus six cycles of MOPP/ABV chemotherapy followed by IFRT or four cycles of MOPP/ABV followed by STNRT. There was no difference in 5-year EFS

(84%-88%) or 10-year OS (84%-88%). The GHSG HD8 study also demonstrated preserved efficacy with reduced toxicity using IFRT compared with extended-field radiotherapy (EFRT) in unfavorable patients (stage IA-IIA with risk factors as defined in the HD10 study, or stage IIB disease with at least three lymph node sites of involvement or an elevated ESR). Patients received COPP (cyclophosphamide, vincristine, procarbazine, and prednisone) and ABVD for two cycles each and were randomized to either 30 Gy of EFRT ($n = 532$) or 30 Gy IFRT ($n = 532$). All patients received an additional 10 Gy of radiotherapy to bulky sites of disease defined as a single lymph node or conglomerate mass measuring $>5\text{cm}$. At a median follow-up of 54 months, the FFTF and OS were 83% and 91%, respectively, and not different between the 2 arms. Toxicity, in terms of myelosuppression and gastrointestinal side effects, were more prominent in the EFRT arm.

The subsequent HD14 study examined the role of intensified chemotherapy in this patient population with the incorporation of the escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) regimen, which was superior to COPP/ABVD in patients with advanced-stage disease (see full discussion in frontline therapy for advanced-stage HL). In the study, 1,528 patients were randomized to standard therapy with four cycles of ABVD versus two cycles of escalated BEACOPP followed by two cycles of ABVD. All patients received 30 Gy of IFRT. The intensified arm resulted in improvement in the primary endpoint of 5-year FFTF at 95% versus 88% ($p < .001$) 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. Second malignancies were 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.

In terms of the role of chemotherapy only in unfavorable patients without bulky disease, the Canadian/ECOG H6 study randomized patients to ABVD alone ($n = 137$) versus ABVD for two cycles followed by STNRT ($n = 139$). At a median follow-up of 11.3 years, the freedom from disease progressive favored the combined modality arm at 94% versus 86% ($P = .006$). OS, however, was superior in the chemotherapy-only arm at 92% compared with 81% ($P = .04$) in the radiation-containing arm because of late deaths in patients receiving radiotherapy.

Stanford V

The Stanford V regimen, which includes mechlorethamine, doxorubicin, vinblastine, prednisone, vincristine, bleomycin, and etoposide, is an alternative approach used in early stage

cHL. In patients with stage I or IIA nonbulky cHL, 87 patients received 8 weeks of Stanford V plus 30 Gy of IFRT, and 61 patients with bulky, early stage disease received 12 weeks of chemotherapy followed by 36 Gy to bulky sites. At a median follow-up of 8 years, the 8-year FFP was 96% in patients without bulky disease and 92% in patients with bulky disease. In patients with bulky mediastinal disease treated in the ECOG 2496 study, 136 received ABVD for six cycles plus 36 Gy, and 131 patients were treated with 12 weeks of Stanford V with 30 Gy of IFRT to sites >5 cm and 36 Gy to the mediastinum. At a median follow-up of 5.5 years, there was no difference in the 5-year failure-free survival (FFS) at 85% versus 77% ($P=.13$) or OS 95% versus 92% ($P=.31$). Toxicity was similar between arms.

Chemotherapy alone

Given the late effects of radiotherapy, including risk of secondary malignancies, especially breast cancer in women <30 years of age and cardiovascular disease, a number of studies have evaluated the use of chemotherapy only. In the Canadian/ECOG H6, the standard therapy arm consisted of STNRT (mantle, spleen plus peri-aortic to 35 Gy in 20 fractions) for standard-risk patients. High-risk patients also received two cycles of ABVD. The experimental arm consisted of four to six cycles of ABVD. Although the study was closed early after the EORTC study demonstrated the superiority of combined modality therapy using IRFT over EFRT, the results remain statistically significant with an improvement in OS in the chemotherapy arm because of late deaths in the radiation arms. The causes of death in the ABVD arm included six deaths due to HL, four due to second cancers, and two related to cardiac disease. In the radiotherapy arms, there were four deaths due to HL, 10 related to secondary malignancies, two due to cardiac disease, three related to infection, and five due to miscellaneous causes. The contribution of the EFRT to late deaths beyond what would be anticipated with modern radiotherapy using IFRT is unclear. There is only a single randomized trial comparing chemotherapy to combined modality treatment in which a proportion of the patients received IFRT. Straus et al. (2004) randomized 152 patients with stage IA-IIIA HL to six cycles of ABVD chemotherapy alone or ABVD with IFRT or modified EFRT. There was no difference in FFP (86% vs. 81%; $P=.61$) or OS (97% vs. 90%; $P=.08$) at 5 years, although the study was closed early for poor accrual and the study was under-powered to detect a difference.

Summary of frontline therapy

For patients with favorable disease, current options include three to four cycles of ABVD plus IFRT, typically 30 Gy, except for patients meeting the criteria for the GHSG HD 10 study, where two cycles of ABVD plus 20 Gy IFRT is an

appropriate option. 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 four cycles of ABVD plus 30 Gy of IFRT. Two cycles of escalated BEACOPP followed by two cycles of ABVD followed by 30 Gy of IFRT, in patients fitting the criteria for the GHSG HD14 study, results in improved disease control, without an OS benefit at the expense of increased toxicity. For patients with bulky disease, options include four to six cycles of ABVD for 12 weeks or Stanford V followed by 36 Gy of IFRT. Chemotherapy alone with six cycles of ABVD is an alternative, especially in young women with a high risk of radiotherapy-related breast cancer, and has a small to result in inferior OS.

Risk-adapted strategies

Recent evidence suggests that interim PET scans are highly predictive of outcome in HL. Gallamini et al. (2007) evaluated 260 patients with stage IIB-IV HL, the majority of whom were treated with ABVD chemotherapy with or without radiation. Patients underwent PET scans after two cycles of therapy. Approximately 20% of patients were PET positive. At a median follow-up of 2 years, the PFS in PET-negative patients was 95%, whereas only 12.8% of patients with a positive PET scan were free from disease ($P<.0001$). The predictive value in patients with early stage disease is less clear. In a study of 77 patients, 5 of 16 interim PET-positive patients had early stage disease and only one patient relapsed. In addition, a retrospective series of 96 patients with nonbulky, early stage disease, end-of-treatment, not interim PET was predictive of PFS at 94% in the PET-negative group compared with 54% in the PET-positive group ($p<.0001$).

Given the prognostic value of interim PET scans in predicting FFS, a number of ongoing clinical trials are using PET-directed risk-adapted therapy. In the United Kingdom RAPID trial, patients with early stage HL undergo imaging with PET scans after three cycles of therapy. PET-negative patients are randomized to receive no further therapy versus IFRT. PET-positive patients receive consolidation with IFRT. The U.S. Intergroup is conducting a trial in patients with early stage, nonbulky disease. Patients who are PET positive after two cycles of ABVD receive two additional cycles without radiotherapy. PET-positive patients receive two cycles of escalated BEACOPP plus radiotherapy. The EORTC is evaluating similar intensified approaches in interim PET-positive patients with favorable and unfavorable disease. Risk-adapted strategies remain experimental at this time.

Toxicity of chemotherapy

In general, ABVD and Stanford V are well tolerated with nausea, vomiting, constipation, alopecia, and peripheral

neuropathy being common. Doxorubicin-related cardiotoxicity in the absence of mediastinal radiotherapy is rare in this patient population, as the total cumulative dose of drug 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. The majority of patients receiving ABVD will develop significant granulocytopenia with anemia and thrombocytopenia being very uncommon. When patients present for chemotherapy, many will have low absolute neutrophil counts, and some patients will be frankly neutropenic. Despite this, retrospective data suggests the risk of febrile neutropenia is very low, at <1% per cycle. The administration of white blood cell growth factors has been associated with an increased risk of bleomycin lung toxicity. The majority of patients may be treated safely with full-dose therapy, on time, without growth factors. For patients who develop febrile neutropenia, neupogen for the minimal number of days to support the white blood cell count should be administered.

Bleomycin-associated pneumonitis is common and presents in ~20% of patients receiving a full course of ABVD chemotherapy. The discontinuation of bleomycin does not appear to adversely affect the efficacy of ABVD chemotherapy. There are not well-studied guidelines for following patients who are receiving bleomycin. Baseline pulmonary function tests should be obtained before chemotherapy. 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 for the presence of basilar crackles and oxygen desaturation with ambulation and/or at rest. Chest x-ray may reveal an interstitial pattern of abnormality and a decline in the diffusing capacity (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. With regard to fertility, the risk of premature ovarian failure with ABVD is very low. Studies evaluating the prophylactic use of gonadotropin-releasing hormone (GNRH) agonists have been equivocal. For patients diagnosed in their 30s who desire to retain fertility, referral to a reproductive endocrinologist may be considered.

Key points

- Patients with early stage cHL are risk stratified according to a number of factors, including 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.

Key points (continued)

- Therapeutic options include combined modality therapy: two to four cycles of ABVD (favorable), four to six cycles of ABVD (unfavorable), or the Stanford V regimen plus IFRT.
- Chemotherapy alone with ABVD in patients without bulky disease is an alternative in selected cases.

Frontline therapy for advanced-stage HL

Clinical case

A 68-year-old man with a history of hypertension and asthma presented with 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, and was consistent with cHL, NS subtype. PET and CT scans demonstrated extensive bilateral cervical, supraclavicular, axillary, mediastinal, hilar, retroperitoneal adenopathy with 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, WBC 15.5, hemoglobin (Hgb) 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 EF of 20%.

ABVD

Since the early 1990s, the treatment of patients with advanced-stage HL has relied on combination chemotherapy with ABVD (see Table 20-3). ABVD was first introduced by Bonadonna et al. (1975) in the late 1970s as an effective, non-cross-resistant chemotherapy option for patients with relapsed HL. Unlike MOPP, the first successful combination chemotherapy regimen used in the treatment of HL, ABVD was not associated with sterility or secondary myelodysplasia or leukemia. The success of ABVD in the second-line setting eventually led to efforts to combine MOPP and ABVD in 12 alternating treatment cycles (MOPP/ABVD alternating regimen). As frontline therapy in patients with stage IV HL, MOPP/ABVD proved superior to MOPP alone with a FFP at 8 years of 64.6% versus 35.9% ($P < .005$) and 8-year OS rates of 83.9% versus 63.9% ($P < .06$). In 1982, the Cancer and Leukemia Group B (CALGB) in the United States initiated a randomized multicenter trial in 361 patients with stage III-IV previously untreated HL, comparing MOPP for six to eight cycles ($n = 123$), ABVD for six to

eight cycles ($n = 115$), and alternating MOPP/ABVD for 12 cycles ($n = 123$). In this trial, complete response (CR) rate (67% MOPP, 82% ABVD, and 83% MOPP/ABVD, $P = .006$) and 5-year FFS (50% MOPP, 61% ABVD, and 65% MOPP/ABVD, $P = 0.02$) were superior in the ABVD and MOPP/ABVD arms, with less neutropenia, thrombocytopenia, and infectious toxicity in the ABVD arm (18%, 2%, and 2% ABVD; 47%, 36%, and 11% MOPP; and 53%, 28%, and 12% MOPP/ABVD, respectively). A subsequent randomized phase III U.S. Intergroup trial compared ABVD and a MOPP/ABV hybrid (combined MOPP and ABV drugs in each cycle rather than alternating cycles of MOPP and ABVD) for 8-10 cycles in 856 patients with stage III-IV HL. CR rates (76% vs. 80%, $P = .16$) and 5-year FFS rates (63% vs. 66%, $P = .42$) were similar in patients receiving ABVD or MOPP/ABV, respectively. In this study, the 5-year FFS for patients with 0, 1, 2, 3, 4, and ≥ 5 risk factors by the IPS (Table 20-4), were 76%, 72%, 81%, 69%, 64%, and 67%, respectively; the 5-year OS was 82% using ABVD; and ABVD resulted in fewer pulmonary and hematologic toxicities, treatment-related deaths, and second malignancies, including acute leukemia, than observed with MOPP/ABV. In a U.K. study comparing ABVD to other combination hybrid regimens (ChlVPP/PABIOE and ChlVPP/EVA), the 3-year EFS and OS with ABVD were 75% and 90%, respectively, once again similar to multidrug regimens, 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; however, recently, two other combination chemotherapy regimens, Stanford V and BEACOPP (see Table 20-3) have challenged the role of ABVD as the standard frontline regimen in this patient population.

Stanford V

In phase II trials with Stanford V administered weekly over 12 weeks (Table 20-3) followed by 36 Gy consolidative radiotherapy to bulky mediastinal disease, tumor masses, or lymphadenopathy ≥ 5 cm, the 5-year FFP was 85%-89% in patients with bulky stage I-II and advanced-stage HL. In a randomized Italian trial in 355 patients with stage IIB, III, and IV HL comparing six cycles of ABVD ($n = 122$), 12 weeks of Stanford V ($n = 107$), and six cycles of MOPPEBVCAD (mechlorethamine, vincristine, procarbazine, prednisone, epoxirubicin, bleomycin, vinblastine, lomustine, doxorubicin, and vindesine), 5-year FFS were 78%, 54%, and 81%, respectively ($p < .01$). In this trial, however, radiation was given only to sites of initial bulky disease or sites of residual disease postchemotherapy, and only 66% of patients receiving Stanford V also received radiation in the Italian study, compared with 91% in the prior single-institution trial. At

Table 20-3 Frontline chemotherapy regimens in HL.

ABVD	Adriamycin 25 mg/ m ²	IV	Days 1 and 15	
	Bleomycin 10 units/m ²	IV	Days 1 and 15	Q28 days
	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	
	Etoposide 100 mg/m ²	IV	Days 1-3	Q21 days
	Adriamycin 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	
	Etoposide 200 mg/m ²	IV	Days 1-3	Q21 days
	Adriamycin 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	Adriamycin 25 mg/m ²	IV	Week 1, 3, 5, 7, 9, 11	
	Vinblastine 6 mg/m ²	IV	Week 1, 3, 5, 7, 9, 11	
	Vincristine 1.4 mg/m ² (capped at 2.0 mg)	IV	Week 2, 4, 6, 8, 10, 12	
	Bleomycin 5 u/m ²	IV	Week 2, 4, 6, 8, 10, 12	
	Mustard 6 mg/m ²	IV	Week 1, 5, 9	
	Etoposide 60 mg/m ²	IV	Week 3, 7, 11	
	Prednisone 40 mg/m ²	PO QOD	Week 1-9, taper by 10 mg QOD week 10 and 11	

IV= intravenous; PO = per os or by mouth; QOD = every other day.

Table 20-4 International Prognostic Score in advanced-stage HL.

Number of risk factors*	5-year FFP	5-year OS
0	84 + 4	89 + 2
1	77 + 3	90 + 2
2	67 + 2	81 + 2
3	60 + 3	78 + 3
4	51 + 4	61 + 4
>5	42 + 5	56 + 5

* The IPS is derived from a multicenter study 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, Hgb <10.5 g/dL, absolute lymphocyte count <600/mm³ or <8% of WBC, albumin <4.0 g/dL, and stage IV disease.

FFP = freedom from progression, OS = overall survival.

From Hasenclever D, Diehl V: A prognostic score for advanced Hodgkin's disease. International Prognostic Factors Project on Advanced Hodgkin's Disease. *N Engl J Med.* 1998;339:1506-1514.

10 years of follow-up, 10-year FFS were 75%, 74%, and 49% ($p < .001$) and 10-year OS were 87%, 80%, and 78% ($P = .4$) for the ABVD, MOPPEBVCAD, and Stanford V arms, respectively; however, in looking at Stanford V patients treated with and without radiotherapy, 10-year disease-free survival (DFS) was statistically in favor of those patients receiving radiation (76% vs. 33%, $P = .004$). In a subsequent multicenter U.K. study in 520 patients with stage IIB, III, IV, or bulky stage I-II HL, there were no differences in 5-year FFS and OS in patients receiving six to eight cycles of ABVD (53% patients received radiation to sites of residual disease) or 12 weeks of Stanford V (73% patients received radiation to disease sites >5 cm or splenic nodules). More pulmonary toxicity was observed in the ABVD arm in this trial, although myelosuppression and neuropathy was slightly worse with Stanford V. ECOG 2496, a randomized phase III U.S. Intergroup trial, demonstrated no significant difference in 5-year FFS (73% vs. 71%, $P = .29$) or 5-year OS (88% vs. 87%, $P = 0.87$) in 812 patients with bulky stage I-II, III, or IV HL receiving six to eight cycles of ABVD with radiation to bulky mediastinal disease or 12 weeks of Stanford V chemotherapy with radiotherapy to disease >5 cm or splenic nodules, respectively. With median follow-up of 5.25 years, 26 second malignancies were observed (14 ABVD and 12 Stanford V). Therefore, as a result of these trials, six cycles of ABVD has remained the standard treatment for patients with advanced stage HL; however, Stanford V may be acceptable in patients for whom a shortened treatment duration or reduction in cumulative doses of bleomycin or adriamycin is desirable.

BEACOPP

The GHSG HD9 trial randomized patients ages 15-65 years with stage IIB, III, and IV HL to either eight cycles of COPP/

ABVD (cyclophosphamide, vincristine, procarbazine, and prednisone alternating with ABVD), BEACOPPbaseline (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone), or BEACOPPescalated (Table 20-3). With 10 years of follow-up, FFTF was 64%, 70%, and 82% with OS of 75%, 80%, and 86% in the COPP/ABVD, BEACOPPbaseline, and BEACOPPescalated arms, respectively. By IPS, FFTF at 5 years was 92%, 87%, and 82% with BEACOPPescalated for patients with 0-1, 2-3, and 4-7 risk factors, respectively. Only 13% of patients receiving BEACOPPescalated had 4-7 risk factors on this trial, however, and although differences in FFTF were significantly in favor of BEACOPPescalated among all three risk groups, statistically significant improvements in OS were observed only in patients with IPS scores of 2-3 ($P = .27$ for IPS 0-1, $P < .0027$ for IPS 2-3, and $P = .16$ for IPS 4-7). When analyzed by age, there was not a significant improvement in FFTF and OS for patients ages 60-65 years and increased toxicity occurred with BEACOPPescalated. Therefore, BEACOPPescalated is not recommended in patients >60 years of age. To reduce toxicity, the HD12 trial examined eight cycles of BEACOPPescalated versus four cycles of BEACOPPescalated plus four cycles of BEACOPPbaseline. Five-year FFTF and OS were 86.4% and 92%, respectively, with eight cycles of BEACOPPescalated compared with 84.8% and 90.3% with the 4+4 arm, and toxicities were not significantly reduced with the 4+4 approach.

In a randomized Italian study comparing six cycles of ABVD with four cycles of BEACOPPescalated plus two cycles BEACOPPbaseline, the 5-year PFS was superior for BEACOPP (81%) compared with ABVD (68%), although there were no differences in OS perhaps because of the smaller numbers of patients (307) on this trial compared with the 1,201 patients on the previous GHSG HD9 trial. In a second Italian cooperative group study, the 7-year rate of freedom from first progression was 85% in patients receiving four cycles of BEACOPPescalated plus four cycles BEACOPP baseline compared with 73% in patients receiving six to eight cycles of ABVD ($P = .004$). In this same trial, the 7-year rate of freedom from second progression was 88% in the BEACOPP group compared to 82% in the ABVD group ($P = .12$), suggesting that long-term outcomes may not differ between the two regimens when one factor in the efficacy of salvage chemotherapy and autologous stem cell transplant following ABVD.

Despite the success of the BEACOPPescalated regimen compared with hybrid regimens and ABVD in patients ≤60 years of age, this regimen is associated with infertility and a 6% second malignancy rate, including a 3.2% incidence of secondary AML or MDS. In addition, BEACOPPescalated is associated with more acute toxicities, including hematologic and infectious complications, than observed with ABVD.

Therefore, although several studies demonstrate the superiority of BEACOPPescalated with respect to PFS in patients with advanced-stage HL, it remains unclear whether the risks of second malignancies, infertility, and acute infections associated with BEACOPPescalated are justified to improve patient outcomes in all patients with advanced-stage HL, especially if relapsing patients after ABVD can be salvaged effectively with high-dose therapy and stem cell transplantation. Currently ABVD, Stanford V, and BEACOPPescalated are all accepted frontline therapies in patients with advanced-stage HL.

Radiation therapy as consolidation in stage III-IV HL

Several studies have examined the role of consolidative radiotherapy in patients with advanced-stage HL, and to date, no study has demonstrated a clear OS advantage with combined modality therapy in patients achieving a CR or partial remission (PR) with chemotherapy alone. The H89 *Groupe d'Etude des Lymphome de l'Adulte* (GELA) study randomized 533 patients with stage III-IV HL to six cycles of MOPP/ABV hybrid or six cycles of ABVPP followed by either two more cycles of chemotherapy or STNRT for patients achieving a CR or PR after six cycles. Ten-year OS was superior in the chemotherapy-alone arms (90% for ABVPP \times 8, 78% for MOPP/ABV \times 8, 82% for MOPP/ABV \times 6 with radiation, and 77% for ABVPP \times 6 with radiation, $P = .03$). Using an ABVD backbone, Laskar et al. (2004) demonstrated an improvement in 8-year EFS and OS with chemotherapy and IFRT in patients achieving a CR; however, this trial was small with only 179 patients randomized to observation versus radiotherapy and included all stages ($n = 80$ with stage III-IV disease). A larger, although nonrandomized, U.K. study restricted to advanced-stage HL patients analyzed outcomes in 807 patients treated with six cycles of ABVD, CHLVPP/PABLOE, or CHLVPP/EVA and IFRT to sites of residual masses or bulky disease. Three hundred patients (43%) received IFRT, and although nonradiotherapy patients tended to be more often in CR, 5-year EFS was 86% with radiotherapy and 71% without radiotherapy ($P < .001$) and 5-year OS was 93% with radiotherapy and 87% without radiotherapy ($P < .001$). In contrast, a randomized study of 739 patients with advanced-stage HL assigned patients with a CR after six to eight cycles of MOPP/ABV hybrid to observation or IFRT, and demonstrated no difference in 5-year OS ($P = .07$) or EFS ($P = .35$) in the radiotherapy ($n = 172$, OS of 85% and EFS of 79%) group compared with the observation group ($n = 161$, OS of 91% and EFS of 84%). The HD12 trial randomized responding patients after BEACOPP with stage IIB, III, and IV HL and with bulk or residual tumor on CT imaging to either

additional consolidative radiotherapy or no radiotherapy. In this trial, 730 patients were randomized to radiotherapy or no radiotherapy and 5-year FFTF was 87% in those patients who did not receive radiotherapy, compared to 90.4% in the radiotherapy arm ($P = 0.08$). Therefore, on the basis of these studies, it appears that consolidation with IFRT does not improve outcomes in patients achieving a CR after combination chemotherapy, although additional studies need to be done in patients with a PR, particularly utilizing a PET-based response determination.

Autologous transplant as consolidation in stage III-IV HL

Several trials have examined the role of autologous transplant to improve outcomes in patients with high-risk, advanced-stage HL, and to date none have demonstrated a role for this following standard ABVD chemotherapy. A European intergroup trial randomized 163 patients with stage III-IV HL and two risk factors (elevated LDH, bulky disease, stage IV with two or more extranodal sites, anemia, or inguinal involvement) achieving a CR or PR after four cycles of ABVD or doxorubicin containing induction (MOPP/ABVD, MOPP/ABV, CVPP/ABV) to either four additional cycles of the same induction chemotherapy or autologous stem cell transplantation after BEAM (carmustine, etoposide, cytarabine, and melphalan) or CBV (cyclophosphamide, carmustine, and etoposide) conditioning regimens. With continued chemotherapy, the 5-year FFS with continued chemotherapy was 82% compared with 75% ($P = .4$) with consolidative autologous transplantation, and the 5-year OS were 88% and 88% ($P = 0.99$), demonstrating no clear benefit from early high-dose consolidation in high-risk advanced-stage HL. Two other studies using hybrid induction chemotherapy regimens versus chemotherapy followed by myeloablative transplant in 126-158 high-risk patients also similarly demonstrated no difference in OS with frontline transplantation.

Future directions and upcoming studies in frontline therapy for stage III-IV HL

In the future, therapy for advanced-stage HL may be tailored based on a patient's pretreatment risk factors (clinical or selected biologic markers) or on results of interim PET or CT. In previous studies, 2-year PFS for patients with a positive PET or CT after two cycles of ABVD were 12.8% compared with 95% for interim PET- or CT-negative patients ($P < .0001$), and interim PET or CT results were more predictive of outcome than IPS score in multivariable analysis. Although the prognostic potential of PET or CT may be dependent on initial treatment, timing of scans, and

definitions of PET negativity, PET or CT does represent an important tool that may direct future therapy. To date, altering therapy based on interim PET or CT results remains investigational; however, a number of trials are ongoing examining this question in patients with advanced-stage HL. Risk-adapted therapy was first described by Dann et al. (2007), who prospectively assigned 108 patients with unfavorable HL (stages I-II with risk factors, B symptoms, bulky disease, or stages III-IV) to either two cycles of BEACOPP-escalated if IPS score >3 or BEACOPPbaseline if IPS score <3 followed by interim PET or CT or gallium scan. Patients with a positive interim scan received four more cycles of BEACOPPescalated, and those with a negative interim scan received four cycles of BEACOPPbaseline. Using this IPS and imaging-directed therapeutic approach resulted in 5-year EFS and OS of 85% and 90%, respectively. Currently, U.K. and U.S. intergroup studies are exploring intensification of therapy in patients who are interim PET or CT positive after two cycles of initial ABVD therapy, whereas the GHSG HD18 trial is reducing therapy after two cycles of initial BEACOPPescalated in patients who are interim PET or CT negative. Central review of interim PET or CT is critical in these studies using PET or CT to alter treatment approaches.

In addition to efforts to risk-stratify therapy based on initial high-risk features or interim PET or CT, efforts also are being made to incorporate promising new therapies into traditional ABVD backbones to further improve outcomes in advanced-stage HL. Brentuximab vedotin, a CD-30 antibody-drug conjugate, which is approved by the U.S. Food and Drug Administration (FDA) for the treatment of relapsed and refractory HL and is further described below, recently has been added to the ABVD regimen. In this phase I trial, patients with stage III-IV HL received ABVD with escalating doses of brentuximab vedotin ranging from 0.6–1.2 mg/kg on days 1 and 15 of each 28-day cycle. Initial toxicities included significant pulmonary toxicity, leading to removal of bleomycin from the regimen and treatment of an expanded cohort of patients with AVD plus brentuximab. Randomized studies in patients with bulky stage II, III, and IV HL comparing the AVD plus brentuximab regimen to traditional ABVD are planned, but this may offer an alternative strategy for improving therapy in high-risk patients using a novel targeted agent and ultimately may become an attractive option in young, high-risk patients with less risk of secondary malignancies or infertility than intensification with BEACOPPescalated. Long-term follow-up will be needed from randomized studies, however, comparing AVD plus brentuximab with ABVD and BEACOPPescalated to determine the efficacy and risks with AVD plus brentuximab before this approach to therapy is accepted in advanced-stage patients.

Key points

- ABVD results in superior CR rates and 5-year FFS when compared with MOPP with less toxicity than MOPP/ABV hybrid or MOPP/ABVD in patients with advanced-stage HL.
- Stanford V (combined chemotherapy over 12 weeks followed by radiotherapy) results in similar 5-year FFS and OS as ABVD in patient with advanced-stage HL.
- Escalated BEACOPP is associated with superior PFS and FFTF in patients with advanced-stage HL and may be considered as frontline therapy for patients <60 years old, particularly patients with four to seven risk factors by the IPS index. The benefit with respect to OS remains unclear, however, as patients failing ABVD often can undergo effective salvage therapy and stem cell transplant, and there is a risk of secondary leukemia and malignancies with escalated BEACOPP.
- The role of consolidative radiotherapy following chemotherapy is controversial in patients with advanced-stage HL treated with ABVD.

Therapy for relapsed or refractory HL

Clinical case

A 32-year-old man presented with stage IVB cHL in 2009 involving the bone marrow, liver, lungs, spleen, and multiple vertebrae. He received six cycles of ABVD with a negative PET or CT after cycles 2 and 4. PET or CT 1 month after cycle 6 demonstrated a new liver lesion and biopsy confirmed HL. He received three cycles of ICE (ifosfamide, carboplatin, etoposide) and again achieved a CR on PET or CT and underwent autologous stem cell transplantation in 2010. One year following transplant, he developed progressive mediastinal and intra-abdominal adenopathy and new pulmonary nodules, and biopsy of a retroperitoneal lymph node by endoscopic ultrasound confirmed recurrent HL. He has received brentuximab vedotin for 10 cycles, achieving 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 it for 16 cycles. He has one brother who is not an HLA match, but he does have several donor options through the NMDP registry.

NMDP = National Marrow Donor Program.

Salvage therapy and autologous stem cell transplant

Salvage chemotherapy followed by autologous stem cell transplant is the standard of care for patients with relapsed or refractory HL. With respect to salvage regimens before autologous transplant, patients typically receive two to three cycles and then proceed to transplant. There are no randomized data on optimal salvage regimens, however, 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), mini-BEAM (carmustine, etoposide, cytarabine, melphalan), and Dexta-BEAM (dexamethasone, carmustine, etoposide, cytarabine, melphalan) (see Table 20-5) with responses ranging from 70% to 90%. Ideally, the salvage regimen chosen should result in a high overall response rate with acceptable toxicity and not impair stem cell mobilization if transplantation is planned.

In 1997, physicians at Stanford University compared outcomes for 60 patients with relapsed or refractory HL treated with a non-cross-resistant combination therapy until maximal response followed by autologous transplant with 103 matched historical patients receiving chemotherapy alone. Four-year OS, EFS, and FFP were 54% versus 47% ($P = .25$), 53% versus 27% ($P < .01$), and 62% versus 32% ($P < .01$), respectively, favoring transplant compared with conventional salvage therapy. The British National Lymphoma Group randomized 40 patients with relapsed HL to either BEAM followed by autologous transplant or mini-BEAM alone, demonstrating a significant PFS benefit ($P = .005$) with transplantation. A larger trial of 161 chemosensitive patients randomized to two cycles of Dexta-BEAM and autologous transplant or two more cycles of Dexta-BEAM demonstrated a 3-year FFTF of 55% with transplantation compared with 34% without transplant. Neither trial, however, demonstrated an OS benefit perhaps because of limited follow-up or small patient numbers.

For patients who are refractory to frontline or salvage therapy, PFS of 25%-38% with high-dose therapy and stem cell transplantation has been reported, suggesting that these patients also benefit from transplantation, although not unexpectedly with inferior outcomes compared with patients who have chemosensitive disease. These studies are limited by differing definitions of refractory disease; the fact that many of the patients received MOPP containing inductions, which is inferior to standard ABVD; and that few patients who did not respond to salvage therapy were transplanted. For example, in 86 patients of whom 91% failed to achieve a CR with frontline hybrid regimens and 9% had disease progression within 3 months of completing frontline therapy, 62% responded to salvage therapy and 24% progressed. All patients did undergo stem cell transplant and 5-year EFS and OS were 25% and 35%, respectively, with response to salvage therapy significantly associated with survival in multivariate analysis. In a retrospective review of 122 patients who failed to achieve a CR after at least one induction combination chemotherapy regimen and who underwent autologous transplant between 1989 and 1995 at centers participating in the Autologous Bone and Marrow Transplant Registry, 56% had a CR or PR with pretransplant salvage and 44% had less than

a PR. Similar to the previous trial, 3-year PFS and OS were 38% and 50%, respectively. A retrospective study from Vancouver also demonstrated 15-year OS of 39% in patients with refractory disease to initial induction therapy, compared with 67% in chemosensitive patients. In a retrospective analysis of 175 patients with stable disease (SD) or progressive disease (PD) to initial induction and who also failed to respond according to CT findings to salvage therapy, 5-year OS and PFS were 36% and 32%. In multivariate analysis, >18 months between diagnosis and autologous stem cell transplantation (ASCT) was favorably associated with OS. Therefore, in patients with primary refractory disease defined either as failure to achieve a CR on CT (not PET/CT) with frontline chemotherapy or a short remission duration who respond to salvage therapy, high-dose therapy with ASCT may lead to prolonged, durable remissions. Fewer data are available in those patients who fail to respond to salvage therapy or patients with refractory disease by PET/CT.

Although the study by Sweetenham et al. (1999) suggested that some patients who are refractory to salvage therapy may benefit from stem cell transplantation, PET/CT may be helpful in guiding treatment approaches for these patients. A number of studies recently have demonstrated the prognostic value of FDG PET/CT pretransplant, with EFS/PFS of 10%-31% in patients who are PET positive compared with 68%-93% for patients with a negative PET/CT before stem cell transplantation. Although using PET/CT to determine eligibility for stem cell transplantation remains investigational, it is reasonable to recommend two to three cycles of salvage chemotherapy, confirmation of response by PET/CT, and then autologous stem cell transplantation in responding patients. For those patients with clearly progressive disease on PET/CT, alternative salvage regimens should be offered and, if patients respond, autologous transplant could be considered. For those patients with improving disease on CT scan, but persistent PET positivity, autologous transplant is still recommended based on previous retrospective data and the possibility of false-positive PET/CT. Further prospective study is needed in this patient population that incorporates centrally reviewed PET/CT into the response assessment and transplant determination.

Tandem transplantation or sequential high-dose therapy has been evaluated with poor risk or refractory disease and remains investigational. In the largest multicenter trial of 247 patients, 105 patients with primary refractory (defined as less than a PR or progression within the first 90 days from induction doxorubicin containing chemotherapy) or at least two risk factors (time to relapse <12 months, stage III-IV at relapse, or relapse within irradiated sites) underwent tandem ASCT, while 95 patients with intermediate risk disease (only one risk factor) received a single transplant. In this study 5-year FFP and OS were 73% and 85%, respectively, in the intermediate-risk group and 46% and 57%, respectively, for

Table 20-5 Salvage combination chemotherapy regimens utilized for relapsed or refractory Hodgkin lymphoma.

GVD (not previously transplanted)	Gemcitabine 1,000 mg/m ²	IV	Days 1 and 8	
GVD (previously transplanted)	Vinorelbine 20 mg/m ²	IV	Days 1 and 8	Q21 days
	Liposomal doxorubicin 15 mg/m ²	IV	Days 1 and 8	
	Gemcitabine 800 mg/m ²	IV	Days 1 and 8	
ICE	Vinorelbine 15 mg/m ²	IV	Days 1 and 8	Q21 days
	Liposomal doxorubicin 10 mg/m ²	IV	Days 1 and 8	
	Ifosfamide 5,000 mg/m ²	IV over 24 hours	Day 2	
DHAP	Mesna 5,000 mg/m ²	IV over 24 hours	Day 2	Q14 days
	Etoposide 100 mg/m ²	IV	Days 1-3	
	Carboplatin AUC=5 (maximum dose of 800 mg)	IV	Day 2	
ESHAP	Dexamethasone 40 mg	IV/PO	Days 1-4	
	Cisplatin 100 mg/m ²	IV over 24 hours	Day 1	Q21 days
	Cytarabine 2,000 mg/m ²	IV every 12 hours	Day 2	
Mini-BEAM	Etoposide 40 mg/m ²	IV	Days 1-4	
	Methylprednisolone 500 mg	IV	Days 1-5	Q21 days
	Cytarabine 2,000 mg/m ²	IV	Day 5	
Dexa-BEAM	Cisplatin 25 mg/m ²	CIV	Days 1-4	
	BCNU (carmustine) 60 mg/m ²	IV	Day 1	Q21-28days
	Etoposide 75 mg/m ²	IV	Days 2-5	
IGEV	Cytarabine 100 mg/m ²	IV every 12 hours	Days 2-5	
	Melphalan 30 mg/m ² (maximum of 50 mg)	IV	Day 5	Q28 days
	Dexamethasone 24 mg	PO	Days 1-10	
GDP	BCNU (carmustine) 60 mg/m ²	IV	Day 2	Q28 days
	Melphalan 20 mg/m ²	IV	Day 3	
	Etoposide 200 mg/m ²	IV every 12 hours	Days 4-7	
ChLVPP	Cytarabine 100 mg/m ²	IV ever 12 hours	Days 4-7	
	G-CSF 300-480 µg	SQ	Day 9 until WBC > 2500/µL	
	Ifosfamide 2,000 mg/m ²	IV	Days 1-4	Q21 days
Brentuximab Vedotin	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	
ChLVPP	Gemcitabine 1,000 mg/m ²	IV	Days 1 and 8	Q21 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	
	Vinblastine 6 mg/m ²	IV	Days 1 and 8	Q28 days
	Procarbazine 100 mg/m ²	PO	Days 1-14	
Brentuximab Vedotin	Prednisone 40 mg	PO	Days 1-14	
	1.8 mg/kg (capped at maximum of 100 kg)	IV	Day 1	Q 21 days

CIV = continuous intravenous; IV = intravenous; PO = per os or by mouth; SQ = subcutaneous.

Source for GVD: Bartlett NL, Niedzwiecki D, Johnson JL, et al. Gemcitabine, vinorelbine, and pegylated liposomal doxorubicin (GVD), a salvage regimen in relapsed Hodgkin's lymphoma: CALGB 59804. *Ann Oncol*. 2007;18:1071-1079. Source for ICE: Moskowitz CH, Nimer SD, Zelenetz AD, et al. A 2-step comprehensive high-dose chemoradiotherapy second-line program for relapsed and refractory Hodgkin disease: analysis by intent to treat and development of a prognostic model. *Blood*. 2001;97:616-623. Source for DHAP: Josting A, Rudolph C, Reiser M, et al. Time-intensified dexamethasone/cisplatin/cytarabine: an effective salvage therapy with low toxicity in patients with relapsed and refractory Hodgkin's disease. *Ann Oncol*. 13:1628-1635. Source for Mini-BEAM: Kuruvilla J, Nagy T, Pintilie M, et al. Similar response rates and superior early progression-free survival with gemcitabine, dexamethasone, and cisplatin salvage therapy compared with carmustine, etoposide, cytarabine, and melphalan salvage therapy prior to autologous stem cell transplantation for recurrent or refractory Hodgkin lymphoma. *Cancer*. 2006;106:353-360. Source for Dexa-Beam: Josting A, Katay I, Rueffer U, et al. Favorable outcome of patients with relapsed or refractory Hodgkin's disease treated with high-dose chemotherapy and stem cell rescue at the time of maximal response to conventional salvage therapy (Dexa-BEAM). *Annals of Oncology*. 1998;9:289-295. Source for IGEV: Santoro A, Magagnoli M, Spina M, et al. Ifosfamide, gemcitabine, and vinorelbine: a new induction regimen for refractory and relapsed Hodgkin's lymphoma. *Haematologica*. 2007;92:35-41. Source for GDP: Kuruvilla J, Nagy T, Pintilie M, et al. Similar response rates and superior early progression-free survival with gemcitabine, dexamethasone, and cisplatin salvage therapy compared with carmustine, etoposide, cytarabine, and melphalan salvage therapy prior to autologous stem cell transplantation for recurrent or refractory Hodgkin lymphoma. *Cancer*. 2006;106:353-360. Source for ChLVPP: Vose JM, Bierman PJ, Anderson JR, et al. CHLVPP chemotherapy with involved-field irradiation for Hodgkin's disease: favorable results with acceptable toxicity. *J Clin Oncol*. 1991;9:1421-1425. Source for Brentuximab Vedotin: Chen R, Gopal A, Smith S, et al. Results from a pivotal phase II study of brentuximab vedotin (SGN-35) in patients with relapsed or refractory Hodgkin lymphoma (HL) [abstract]. *J Clin Oncol*. 2011;29:8031.

those in the high-risk group who underwent tandem transplant, which compares favorably to historically observed 3- to 5-year PFS of 25%-39% in primary refractory patients. In a second trial, however, use of sequential high-dose conditioning regimens (ie, high-dose cyclophosphamide, methotrexate, and etoposide followed by BEAM and then transplant) did not improve FFTF or OS over standard BEAM and transplant. Therefore, single versus tandem transplant and the use of sequential high-dose conditioning regimens remain investigational in patients with relapsed HL.

Therapeutic options for patients relapsing after autologous stem cell transplantation

Although many of the previously discussed combination salvage regimens, including ICE, GVD, DHAP, ESHAP, GDP, and IGEV, have significant activity in patients with HL that has progressed after autologous stem cell transplant, the goals in this patient population often are palliation, with minimization of symptoms as well as treatment-related toxicity. Furthermore, prolonged therapy over several months rather than two or three cycles may be necessary to control disease. Therefore, single-agent regimens often are preferred, and combination regimens are reserved for patients with organ involvement or significant disease-related symptoms. A number of single-agent regimens can be utilized in this 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%-50% of patients. Combination therapy with ChlVPP (chlorambucil, vinblastine, procarbazine, and prednisone) also has activity in this patient population. Selected patients with nonbulky lymphadenopathy and no organ involvement who are otherwise asymptomatic also could be observed in this setting.

Radiotherapy should be considered in the setting of relapsed HL. In a retrospective analysis of salvage radiotherapy used in 100 patients at first treatment failure, typically after COPP/ABVD initial therapy, 5-year FFTF and OS were 28% and 51% with radiotherapy alone. Advanced stage at relapse and B symptoms adversely affected OS in multivariate analysis. Therefore, in highly selected patients with limited stage disease at relapse who may not be eligible for autologous transplantation due to age and comorbid conditions, IFRT may lead to prolonged remissions. For younger patients with relapsed HL, because of potential risks of second malignancies within the radiation field and improved survival with autologous stem cell transplantation, radiotherapy alone is not recommended at first relapse. IFRT, however, should be considered in these patients as consolidation post-autologous

transplant to bulky, nonirradiated sites or to sites of relapsed limited stage disease in previously nonirradiated fields. For those patients with limited stage relapse posttransplantation, radiotherapy may lead to prolonged remissions and may delay need for palliative chemotherapy.

Brentuximab vedotin (Adcetris, SGN-35)

Recently, the FDA approved brentuximab vedotin, a novel anti-CD30 drug-antibody conjugate for the treatment of patients with relapsed or refractory HL after previous stem cell transplant. Brentuximab vedotin is composed of a CD30 antibody conjugated by a plasma-stable linker to the antimicrotubule agent, monomethyl auristatin E (MMAE). In phase I testing, the maximum tolerated dose was 1.8 mg/kg every 3 weeks with an overall response rate of 38% in 45 patients, including 11 complete responses. In a pivotal phase II study with 102 patients with relapsed (29%) or refractory (71%) HL who previously had received a median of 3.5 prior therapies (range 1-13), the overall response rate (ORR) was 75% with a 34% CR rate. The median duration of response was 20.5 months in this trial and grade 3-4 toxicity consisted of sensory neuropathy (8%), neutropenia (20%), and thrombocytopenia (8%). Therefore, with its significant single-agent activity and tolerability, brentuximab vedotin should be considered as initial therapy for patients with relapsed HL post-autologous transplantation. Brentuximab vedotin may be administered for up to 16 cycles, with dose reductions or delays if needed for myelosuppression or neuropathy.

Allogeneic transplant

Allogeneic transplant has been used for patients with relapsed HL after prior autologous transplant, although the presence of a graft-versus-Hodgkin lymphoma effect remains controversial. Most trials of allogeneic transplant in HL demonstrate 2-year PFS rates of 30% and OS of 35%-60%. A European Blood and Marrow Transplantation trial compared reduced intensity ($n = 89$) to myeloablative ($n = 79$) allogeneic stem cell transplant. In this trial, with reduced-intensity conditioning, 1-year treatment-related mortality (TRM) was 23% and 5-year OS was 28%, compared with 46% 1-year TRM and 5-year OS of 22% with myeloablative conditioning. Five-year PFS in the reduced intensity group was 18%. Other prospective and retrospective studies have reported 2- to 3-year PFS of 25%-32% and OS of 43%-64% with reduced-intensity conditioning. Overall, these studies and others demonstrate that in selected patients with available donors, reduced-intensity allogeneic stem cell transplantation is an option for patients with relapsed or refractory HL after prior autologous transplantation and may lead to prolonged DFS in 18%-32% of patients.

Key points

- Salvage chemotherapy followed by autologous transplant offers superior PFS compared with chemotherapy alone in patients with relapsed, chemosensitive HL.
- Selected patients with chemorefractory HL may benefit from autologous transplantation; particularly if they respond to salvage therapy or achieve a negative PET or CT before transplantation.
- Brentuximab vedotin leads to overall response rates of 75% in patients with relapsed HL following autologous transplant. Additional options for patients relapsing after autologous transplant include close observation if asymptomatic and no organ compromise; combination regimens in Table 20-5; single agents like gemcitabine, vinorelbine, or vinblastine; or radiotherapy.

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 with 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 followed only as needed with a primary care physician (PCP). About 9 years postdiagnosis, 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 troponins continued to rise and the patient was urgently taken to cardiac catheterization, demonstrating a 90% occluded left-anterior descending artery.

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, including 54% due to HL, 22% from second malignancies, and 9% from cardiovascular disease. The likelihood of HL recurrence declined after 5 years, whereas the incidence of second malignancies and cardiovascular disease continually increased beginning 10-15 years from the start of treatment. Within the first 5 years from diagnosis, patients typically are 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. Limited data exist, however, regarding the

utility of routine blood work in detecting relapsed disease, and in one series, relapse was detected in 55% of patients by history, 23% by chest x-ray, and 1% by laboratory findings. In a second study of 107 patients, 22 patients relapsed and 64% were detected clinically, 9% detected by laboratory testing, and 9% by CT imaging. With respect to imaging studies, although the convention has been to recommend CT of initially involved sites every 6-12 months during the first 5 years from diagnosis, several previous studies have demonstrated no survival benefit with routine CT surveillance and it does not appear to be cost effective. A more recent examination of follow-up PET/CT demonstrated a high false-positive rate, with an overall positive predictive value of only 28%, limiting its utility as a follow-up tool for HL. In this study, 161 patients had 299 routine or clinically indicated follow-up PET/CTs (defined as PET/CT performed based on clinical suspicion) and in this setting the true positive rates were only 5% and 13%, respectively. Therefore, with the low risk for relapse in most patients with HL, and no demonstrated survival benefit with routine surveillance CT or PET/CT, follow-up should consist of history and physical exam with only symptom-directed imaging during the first 5 years after HL diagnosis. The risks of routine laboratory and imaging studies, including false-positive findings, should be discussed with the patients and the use of routine imaging should be limited without clinical suspicion. Routine surveillance PET/CT currently is not recommended based on low-positive predictive value and associated costs of this testing and workup of false-positive results.

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 even beyond 30 years from diagnosis. Therefore, monitoring of late complications is a lifelong endeavor for HL survivors. In a meta-analysis, second cancers were more commonly encountered in patients receiving chemoradiation or radiation alone compared with chemotherapy alone, and no significant differences in the second malignancy rate were observed with IFRT versus EFRT. Therefore, any patient receiving previous radiotherapy should be monitored for second malignancy and cardiovascular disease. Specifically, annual breast screening (typically mammography) is recommended 8-10 years after completion of treatment or at the age of 40, whichever comes first, in women who received chest or axillary radiation. The risk of secondary breast cancers is associated with young age at the time of radiation, and women <30 years of age are particularly at risk. Lung cancer risk is increased in patients receiving mediastinal radiation, particularly patients with 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, pericardial disease,

cardiomyopathy, and valvular disease, also is observed in HL survivors, particularly after mediastinal radiation or anthracycline-based chemotherapy, starting about 5 years after treatment and also is associated with age at treatment. Although optimal screening strategies are unclear, monitoring and aggressive management of cardiovascular risk factors, including smoking, hypertension, diabetes, and hyperlipidemia, is recommended along with consideration of a baseline stress test or echocardiogram.

Other late toxicities associated with radiotherapy include hypothyroidism, which can occur in up to 50% of patients, and radiation pneumonitis or lung fibrosis, which is fairly uncommon occurring in 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.

With respect to secondary MDS and leukemia, this is not associated with ABVD chemotherapy, but it can be observed in up to 3.2% of patients treated with eight cycles of BEACOPP escalated. It remains to be seen with prolonged follow-up whether efforts to reduce the total cycles of BEACOPP using PET-directed approaches or to alternate escalated and baseline BEACOPP will reduce this secondary leukemia rate. Patients treated with BEACOPP or who have received MOPP, however, should have a CBC monitored annually for this risk. Last, with respect to fertility, several studies have demonstrated no impact of ABVD on gonadal function, and most patients are able to maintain their fertility; however, patients treated with MOPP or BEACOPP typically are infertile and should be counseled about this risk and referred for sperm banking or reproductive endocrinology evaluation if interested before treatment.

Finally, in addition to these risks, patients who undergo autologous stem cell transplant for relapsed disease should be monitored for risks of secondary leukemia, other secondary malignancies, hypogonadism, and its complications, including declines in bone mineral density, and also should be considered for revaccination. In a retrospective study of 153 patients treated with autologous stem cell transplant for relapsed HL, the relative risk of second malignancies was 6.5 compared with the general population and 2.4 compared with nontransplanted patients with HL. Second malignancies occurred in 15 patients, at a median of 9 years posttransplantation and consisted of AML or MDS ($n = 6$), non-HL ($n = 3$), non–small cell lung cancer ($n = 2$), colon cancer ($n = 2$), gastric cancer ($n = 1$), and adenocarcinoma of unknown primary ($n = 1$). All patients with AML or MDS had MOPP as part of their initial treatment regimen. In 100 patients treated with autologous stem cell transplantation in Vancouver, second malignancies occurred in seven patients at a median time of 4.2 years from transplantation, and consisted

of AML or MDS, glioblastoma, renal cell carcinoma, colon carcinoma, non-HL, and breast cancer. In this series, five patients developed cardiovascular disease, including myocardial infarctions 4–11 years posttransplant, arrhythmia, and aortic stenosis.

Last, in the largest retrospective series of outcomes post–autologous transplantation, 16 of 494 patients who underwent autologous stem cell transplantation for relapsed or refractory disease developed second malignancies, and second malignancy was associated with use of total body irradiation (TBI) as part of conditioning, age ≥ 40 years, or use of radiation before transplantation. Therefore, all survivors of HL who are transplanted should be monitored lifelong for second malignancies, with close attention to those treated with radiation either pre- or posttransplant or with TBI during transplant conditioning. In addition, patients typically experience hypogonadism posttransplantation 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 influenza* 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.

Key points

- Routine follow-up for HL survivors consists of history and directed physical examination with symptom directed laboratory testing or imaging. Surveillance CT, PET or CT, and laboratory testing have not been shown to improve survival or 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.

Pediatric HL

HL represents 7% of childhood cancers and is rare in children under the age of 10 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%–15%. LD is rare, except in association with HIV.

The vast majority of pediatric patients with HL are cured of 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 includes patients with stage III and IV disease, bulky mediastinal disease, and all patients with B symptoms.

In a recent study of 88 children with favorable disease, patients received four cycles of VAMP (vinblastine, adriamycin, methotrexate and prednisone). Patients who achieved a complete response by PET scan after two cycles of therapy received no radiotherapy, and those with less than a complete response received 25.5 Gy of IFRT. Overall, the 2-year EFS was 90.8% and there was no difference between those who did not receive and those who received radiotherapy (89.4% vs. 92.5%, $P = .61$). In a study of 216 intermediate- and high-risk children, patients received three cycles of ABVE-PC (adriamycin, bleomycin, vinblastine, etoposide, prednisone, cyclophosphamide). Early responders received 21 Gy of radiotherapy. Slow early responders received two additional ABVE-PC cycles before radiation. Five-year EFS was 84% (86% for the early responders and 83% for the slow early responders, $P = .85$). Five-year OS was 95%. Other approaches in children include the Stanford V regimen with low-dose IFRT and COPP/ABV to limit the exposure to both alkylators and adriamycin.

Nodular lymphocyte predominant HL

Clinical case

A 19-year-old college lacrosse player presented with left-sided cervical adenopathy and 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 and bone marrow biopsy was negative.

*n*LPHL is an uncommon subtype of HL, representing about 5% of cases, with unique pathologic features distinguishing it from cHL. The neoplastic cell is a large cell, the LP cell, otherwise known as a popcorn cell because of its single, large-folded, or multilobulated nucleus (Figure 20-1). Unlike the classic HRS cell, these cells are typically CD30 and CD15 negative, with CD19, CD20, CD45, and CD79a positivity

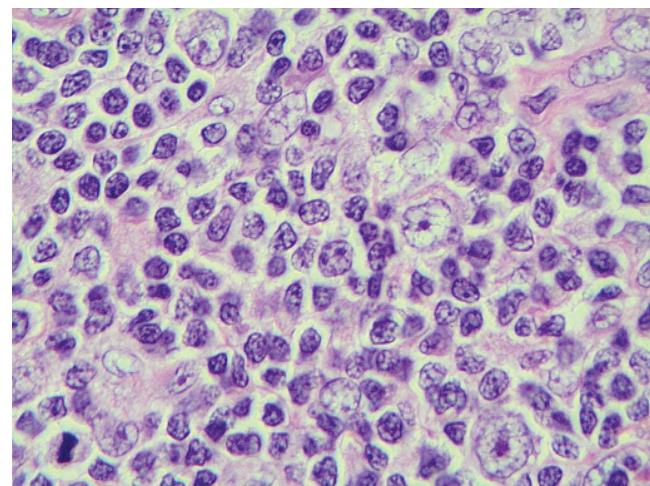


Figure 20-1 LP or popcorn cells in *n*LPHL with typical folded, multilobulated nucleus.

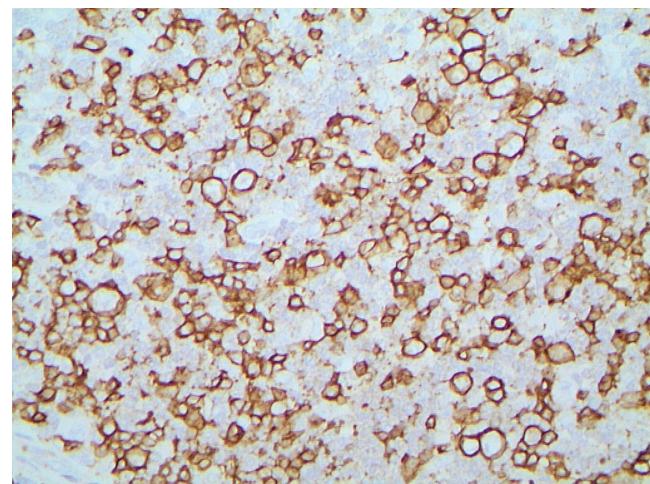


Figure 20-2 CD20 staining on large LP cells in nodular lymphocyte predominant HL.

(Figure 20-2). These cells are also PAX-5 and OCT-2 positive. The surrounding background lymphocytes are predominantly small CD20 B-cells, with rare eosinophils, neutrophils, or plasma cells (Figure 20-3). Surrounding the LP cells, CD4+ T-cell rosettes are found and CD21-positive follicular dendritic cells are present, consistent with the germinal center derivation of this malignancy.

Because of the rare occurrence of this malignancy, presentation, treatment, and patient outcomes are not well described in this disease. In a retrospective analysis of 8,298 patients enrolled on clinical trials for HL through the GHSG, 394 patients had *n*LPHL. In this series, the median age at diagnosis was 37, 75% patients were male, and 79% of patients had early stage disease. Clinically, there appears to be two age peaks, one in children and another in patients ages 30-50 years. The presence of

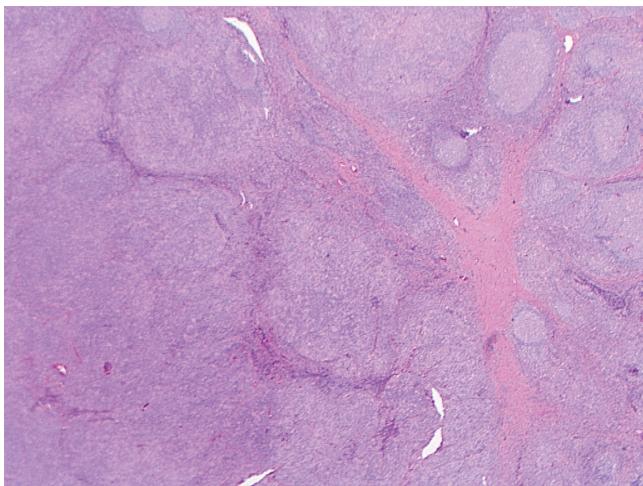


Figure 20-3. Low-power view of *nLPHL*.

B symptoms or bulky disease is unusual, 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. This entity may be observed before, simultaneous with, or following a diagnosis of *nLPHL*. This entity is thought to be a benign condition, but as it occurs concurrently or following a diagnosis of *nLPHL*, biopsy of recurrent adenopathy always is required in this disease to confirm relapse. Likewise, T-cell-rich B-cell lymphoma and *nLPHL* can occur simultaneously or in succession, and frequently T-cell-rich B-cell lymphoma can be confused pathologically for *nLPHL*. With T-cell-rich B-cell lymphoma, large atypical B-cells that are CD20 positive are surrounded by an abundant background of T-cells and histiocytes. Because ~5% of *nLPHL* eventually develop NHL, including T-cell-rich B-cell NHL or diffuse large cell lymphoma, biopsy of recurrent lymph nodes is necessary to determine therapy at relapse. In a series of 22 patients treated with rituximab for *nLPHL*, nine patients relapsed, including five who underwent biopsy at recurrence and, of these, two had diffuse large cell NHL within 13 months of follow-up.

With respect to treatment for *nLPHL*, no standard front-line or relapsed therapy exists, although a number of options are available. In the large GHSG series, outcomes for these

patients appear to be excellent, with ORR of 85%-91% in patients with early stage *nLPHL* compared with ORR of 83%-86% in patients with early stage cHL treated with the same regimens. For advanced-stage patients, outcomes are similarly good with ORR of 78% in patients with NLPNL compared with 78% in patients with stage III-IV classical HL. FFTF at 50 months was 88% in patients with *nLPHL* and 82% for patients with cHL. Interestingly, late relapses >1 year after therapy are observed more commonly in patients with LPHL (7.4%) compared with cHL (4.7%). Adverse prognostic factors in LPHL include advanced stage, hemoglobin <10.5 g/dL, age ≥45 years, and lymphopenia (<8% of total white cell count).

For early stage *nLPHL*, typically IFRT alone or in combination with rituximab or chemotherapy is recommended. Given the excellent long-term survivals of these patients, however, the risks of late secondary malignancies and cardiovascular disease must be considered. In a single institution study of 113 patients with stage I-II *nLPHL*, 93 patients were treated with radiation alone, 13 patients received combined modality therapy, and seven received chemotherapy alone. Twenty of 106 patients receiving radiotherapy relapsed (19%), compared with six of seven patients treated with chemotherapy alone, and 10-year PFS for stage I and II patients treated with radiotherapy were 89% and 72%, respectively. No differences were observed in patients treated with extended-field, regional, or limited-field radiotherapy, and the use of combined modality therapy did not improve PFS or OS over radiotherapy alone. However, 12 patients receiving radiotherapy did develop second cancers, five of which were fatal. The GHSG evaluated 131 patients with stage IA LPHL treated with extended-field ($n = 45$), involved-field ($n = 45$), and combined modality treatment ($n = 41$), and found an FFTF rate of 95% and OS of 99% at 43 months, with no differences with respect to FFTF or OS among the three treatment arms. In this series, only one secondary cancer was observed—that is, gastric carcinoma in the extended-field group. In a retrospective evaluation from the Australasian Radiation Oncology Group of 202 patients with stage I-II *nLPHL* treated with radiotherapy alone, the 15-year FFP and OS were 83% and 82%, respectively. Second malignancies included non-HL ($n = 9$), acute leukemia ($n = 1$), and 18 carcinomas, including breast, lung, gastrointestinal, melanoma, and unknown primary carcinomas. Deaths resulting from cardiac and respiratory complications after radiotherapy occurred in nine patients. In contrast to these three trials demonstrating excellent PFS and OS with radiotherapy alone in early stage *nLPHL*, a retrospective comparison of 32 patients treated with radiotherapy alone versus 56 patients receiving combined modality therapy with ABVD

for two cycles and radiotherapy demonstrated improved PFS survival (65% vs. 91%, $P = .0024$) with combined modality therapy as compared with radiation alone. Therefore, although most series support favorable outcomes with radiotherapy alone in early stage *nLPHL* with the exception of late second malignancies and cardiovascular disease, at least one series advocates treating these patients similar to current cHL treatment approaches with combined modality therapy, and both approaches are standardly utilized. Because of the risks of second malignancies and the excellent long-term outcomes observed in patients with LPHL, in selected patients for whom the disease is completely resected, observation may be a suitable alternative to IFRT.

Chemotherapy typically is reserved for those patients with advanced-stage disease or for whom the risks of late complications of radiotherapy are increased because of field or dose of radiotherapy required. In early stage patients, for whom the risks of secondary malignancies with radiotherapy are of concern, chemotherapy alone occasionally is utilized. For example, a study conducted in the United Kingdom and France prospectively examined the results of three cycles of cyclophosphamide, vinblastine, and prednisolone (CVP) in children and adolescents with stage I-II *nLPHL*. In this trial, the ORR was 100% and the 40-month FFTF and OS were 75% and 100%, respectively. In a study of single-agent rituximab as frontline therapy in 28 patients with stage IA *nLPHL*, the ORR was 100% and at 36 months the PFS was 81%. Therefore, although the FFTF may be slightly lower with chemotherapy alone compared with radiotherapy or combined modality therapy, these early stage patients who relapse can be effectively salvaged with additional chemotherapy and radiotherapy, and such an approach may reduce the rates of second malignancies.

In the advanced-stage setting, chemotherapy options include six cycles of ABVD, alkylator regimens (CVP or CHOP), or rituximab. Because of the CD20 expression on LP cells, rituximab increasingly is utilized in the frontline treatment of advanced-stage *nLPHL*. In one of the initial series examining the activity of this agent, Ekstrand et al. (2003) reported an ORR of 96% in 22 patients, 12 with previously untreated *nLPHL*; however, response duration is limited, with a median FFP of 9.2 months. With the notable activity of single-agent rituximab in relapsed and previously untreated HL, it is frequently combined with ABVD or alkylator-based (CHOP or CVP) therapy as part of initial treatment with those for stage III-IV disease. Limited data exist comparing ABVD to alkylator therapy either alone or in combination with rituximab. Therefore, these regimens frequently are utilized as frontline and salvage therapy for relapsed-stage III-IV *nLPHL*.

Key points

- *nLPHL* cells typically lack CD30 and CD15 staining and are CD19, CD20, CD45, and CD79a positive.
- *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, so biopsy at relapse is necessary.
- Unlike HL, *nLPHL* is associated with noncontiguous nodal spread and late relapses. No standard therapy exists for *nLPHL* and includes IFRT, combined modality therapy, or observation for early stage disease and combination chemotherapy with rituximab or single-agent rituximab for advanced-stage disease.

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Non-Hodgkin lymphoma

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CHAPTER
21

Non-Hodgkin lymphoma

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Overview of lymphocyte development

Lymphocytes develop from a common lymphoid progenitor cell. B-cells mature primarily in the bone marrow, whereas T-cells mature in the thymus. Although the process and signals of maturation differ between the two cell types, they rely on similar genetic events to generate specific antibodies or cell surface receptors. These gene rearrangements are critical for the development of a broad immune repertoire and also provide molecular markers of clonality that can be used to diagnose lymphoid malignancies.

B-cell development

B-cell maturation consists of early (antigen-independent) and late (antigen-dependent) stages. Early development is initiated by the rearrangement of genes for the heavy and

light chains of antibodies, a process referred to as V/(D)/J recombination. The earliest B-precursor cell shows rearrangement of the immunoglobulin heavy chain, which is then followed by light-chain rearrangement. The κ light-chain genes rearrange first; if neither κ locus is productively rearranged, then the λ gene loci undergo rearrangement. Once a successful light-chain rearrangement occurs, the cell will express the complete immunoglobulin molecule on its surface, which identifies it as a mature B-cell. Mature B-cells typically will express either immunoglobulin M (IgM) or immunoglobulin D (IgD) on their surface, and this surface expression is critical to cell survival. Further, the combination of the VDJ gene sequences is unique for each immunoglobulin molecule variable region and, hence, each B-cell and is referred to as the idiotype.

Cell surface markers also are used to define the early stages of B-cell development. CD10 (CALLA, the common acute lymphoblastic leukemia [ALL] antigen) and CD19 are expressed on immature B-cells (pro-B and B-precursor) that have begun heavy-chain rearrangement (Figure 21-1). Terminal deoxynucleotidyl transferase (TdT), a DNA polymerase important for nucleotide chain elongation during gene rearrangement, also is expressed at this stage. CD20 is then expressed as cells rearrange light chains and express surface immunoglobulin, and the expression of CD10 and TdT is lost. The mature but antigen-naïve B-cell leaves the bone marrow to circulate and populate lymphoid organs, such as lymph nodes, spleen, and mucosa-associated lymphoid tissue (MALT).

The late, or antigen-dependent, stages of B-lymphocyte development begin when a naïve B-cell recognizes an antigen with its membrane-bound antibody. These B-cells collect in germinal centers of the various lymphoid organs and begin to divide and undergo several types of genetic modification. Somatic hypermutation is a process by which cells introduce mutations into the variable region (V) genes.

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Off-label drug use: Dr. Savage: Rituximab for follicular lymphoma and management of PTLD. Fludarabine and mitoxantrone as primary therapy for indolent lymphomas; bendamustine as primary therapy for indolent lymphomas and mantle cell lymphoma; etoposide in DLBCL, PMBCL and BL (DA-EPOCHR). GA-101 in DLBCL; brentuximab vedotin in cutaneous ALCL; and alemtuzumab, gemcitabine and rituximab for PTCLs. L-asparaginase in NK/TCLs. Dr. Kahl: Rituximab for follicular lymphoma and management of PTLD. Fludarabine and mitoxantrone as primary therapy for indolent lymphomas; bendamustine as primary therapy for indolent lymphomas and mantle cell lymphoma; etoposide in DLBCL, PMBCL and BL (DA-EPOCHR). GA-101 in DLBCL; brentuximab vedotin in cutaneous ALCL; and alemtuzumab, gemcitabine and rituximab for PTCLs. L-asparaginase in NK/TCLs.

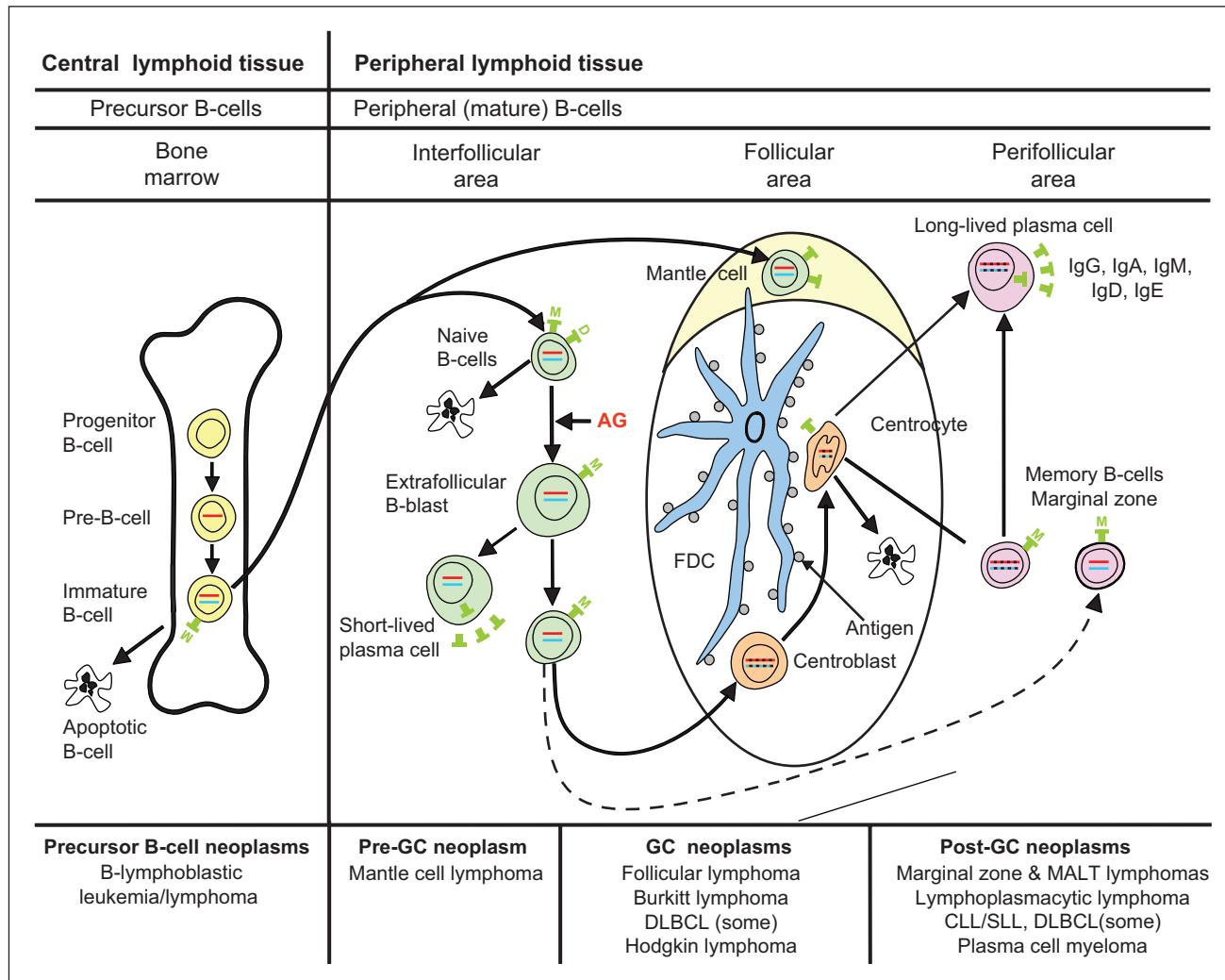


Figure 21-1 Schematic representation of B-cell differentiation (WHO 2008). CLL/SLL = chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL = diffuse large B-cell lymphoma; GC = germinal center; MALT = mucosa-associated lymphoid tissue. Reproduced with permission from Harald Stein.

These mutations result in antibodies that may have a higher or lower affinity for the antigen. Those that produce a higher affinity antibody will persist and become either plasma cells or memory B-cells, whereas those that fail to produce functional antibody at this stage will undergo apoptosis. Class switching involves changing the heavy chain that is expressed to produce other antibody classes: immunoglobulin G (IgG), immunoglobulin A (IgA), or immunoglobulin E (IgE). Although this switch does not alter antibody affinity, the change in class of antibody will alter its effector function and thereby affect the immune response.

T-cell development

T-cell maturation occurs in the thymus, where T-cell precursor–thymic stroma interactions guide the maturation and selection of mature T-cells (Figure 21-2). The differentiation steps of the T-lymphocyte are in many ways

parallel to those of B-lymphocytes. The four T-cell receptor (TCR) genes, α (alpha), β (beta), γ (gamma), and δ (delta), undergo rearrangement analogous to that seen in the immunoglobulin gene locus. These proteins form heterodimeric receptors in mature lymphocytes, and any given T-cell expresses either an $\alpha\beta$ or a $\gamma\delta$ TCR on its surface, but not both. Once a TCR is expressed on the surface of the developing thymocyte, the cell undergoes both positive and negative selection. Positive selection requires the TCR to recognize a self-peptide major histocompatibility complex (MHC) molecule, and negative selection then ensures that the TCR–MHC binding affinity is not high, which could indicate an autoreactive clone. Cells that survive positive and negative selection then exit the thymus as mature T-cells.

In addition to the TCR, other surface molecules expressed on mature T-cells include the CD3, CD4, and CD8 proteins. The TCR is expressed in association with the CD3 antigen, expression of which is considered to be the definitive marker

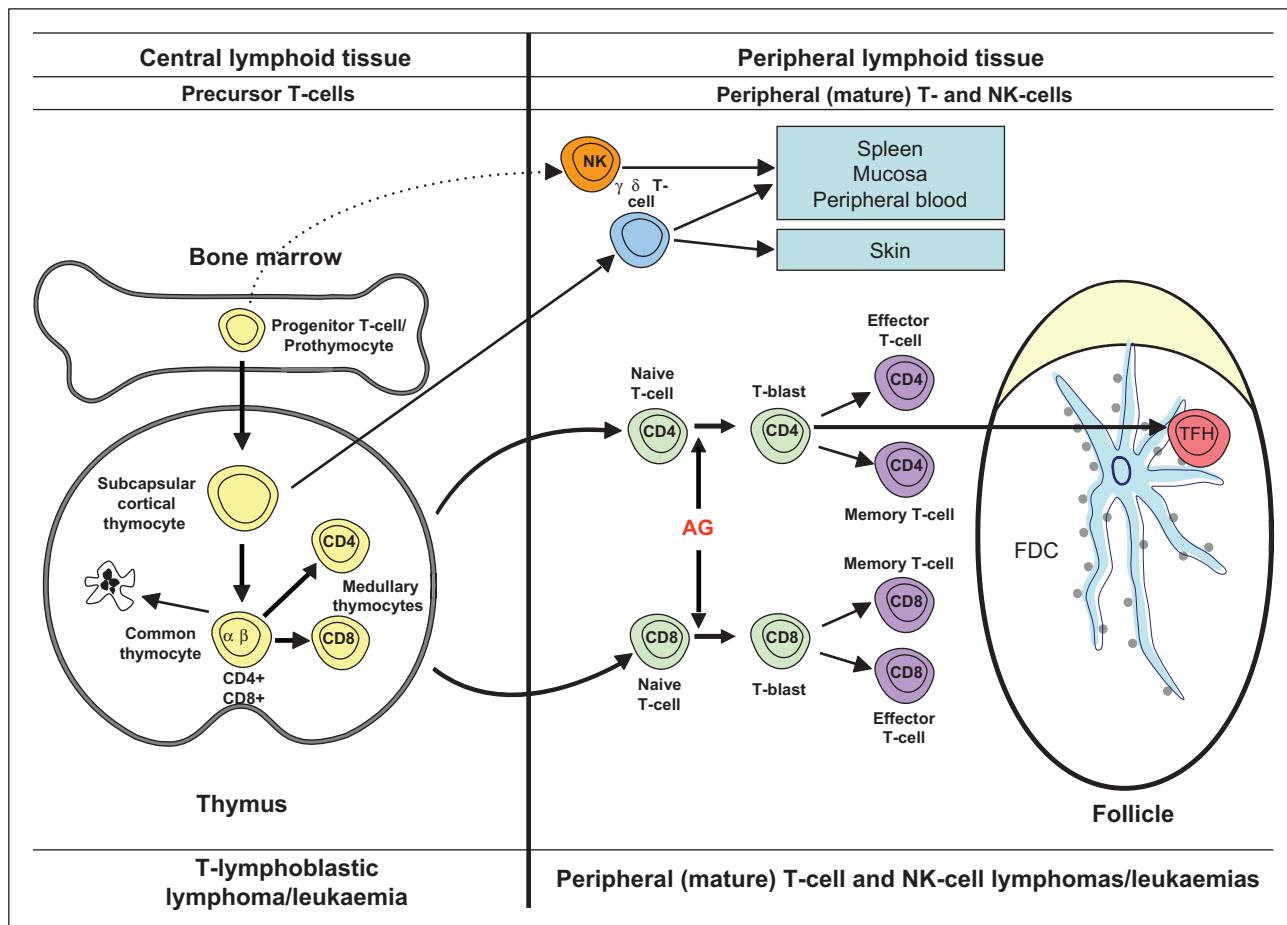


Figure 21-2 Schematic representation of T-cell differentiation (WHO 2008). FDC = follicular dendritic cells; NK = natural killer; TFH = T-helper follicular cells. Reproduced with permission from Harald Stein.

of T-cell identity. Coreceptors with the TCR–CD3 complex are CD4 and CD8, which identify helper and cytotoxic-suppressor subtypes, respectively. Most mature T-cells will express either CD4 or CD8, although occasional cells express both. In addition, virtually all T-cells express the pan-T-cell marker CD5; this same marker is expressed on a subset of normal and malignant B-cells, such as chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Absence of CD5 expression on circulating lymphocytes with some characteristics of T-cells is a marker of natural killer (NK) cells; however, it also can be an important marker for lymphoproliferation because many neoplasms of phenotypically mature T-cells lack CD5 expression.

Biology of lymphomas

The transforming events in lymphoproliferative disorders are best understood for B-cell lymphomas. Analysis of V genes from a variety of B-cell neoplasms reveals evidence of somatic mutation, which indicates that they arise from germinal-center or post-germinal center B-cells. This suggests

that the germinal center is a site of initiation of the transforming event, as cells undergo a number of DNA-modifying events, including class switching and somatic hypermutation. Failure of these processes may result in inappropriate translocations (oncogene to immunoglobulin switch region) or point mutations in oncogenes, resulting in unregulated oncogene activation. Similar errors in DNA rearrangement can occur during V(D)J recombination, resulting in precursor B- or T-cell malignancies. Several viral infections also are associated with the development of lymphoproliferative disorders, including Epstein-Barr virus (EBV) and human herpesvirus 8 (HHV-8) (Table 21-1).

Diagnostic testing in lymphoproliferative disorders

In general, there are no surface markers that are diagnostic of malignancy in lymphocytes. Several methods are used to document a lymphoid malignancy, including morphology (lymph node, peripheral blood, or bone marrow), immunophenotyping, molecular genetics, and cytogenetics.

Table 21-1 Risk factors in the development of non-Hodgkin lymphoma.

Viral	EBV, HTLV-1, HHV-8, hepatitis C
Bacterial	<i>Helicobacter pylori</i>
Impaired/altered immunity	Ataxia-telangiectasia
Congenital	Wiskott-Aldrich syndrome
Acquired	Severe combined immunodeficiency AIDS (HIV infection) Organ or stem cell transplantation Aging Autoimmune and rheumatologic disease
Environmental or occupational	Herbicides Pesticides

AIDS = acquired immunodeficiency syndrome; EBV = Epstein-Barr virus; HHV-8 = human herpesvirus 8; HIV = human immunodeficiency virus; HTLV-1 = human T-cell lymphotropic virus 1.

Morphology

Many lymphoproliferative malignancies are diagnosed by characteristic morphology of a lymph node or other biopsy. Examination of patterns of growth, degree of cytologic atypia, degree and type of differentiation, and the presence of reactive components are important for diagnosis. Fine-needle aspiration can be used to identify an abnormal population of cells, but it furnishes none of the structural information provided by a core or excisional biopsy. For that reason, an excisional biopsy is recommended for the initial diagnosis of a suspected lymphoproliferative disorder.

Immunophenotyping

Diagnosis by immunophenotyping is based on finding increased expression of a certain marker (or markers) that usually is present only on a small percentage of normal cells. For B-cell malignancies, clonality can 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 either κ- or λ-expressing B-cells. This would not be expected in a reactive process. Some pan-B-cell surface markers frequently are coexpressed, such as CD19, CD20, and CD22. Others, including CD5, CD10, and CD23, are helpful in the differential diagnosis of B-cell neoplasms, such as differentiating CLL and small lymphocytic lymphoma (SLL; CD5+, CD19+, CD23+) from follicular lymphoma (FL; CD5-, CD10+, CD19+) or MCL (CD5+, CD19+, CD23-) (Table 21-2).

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 or a pan-T-cell marker 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 involve isolating the DNA from a sample and subjecting it to Southern blot analysis or polymerase chain reaction (PCR) to detect rearrangements of immunoglobulin or TCR genes. The demonstration of a dominant rearrangement of the immunoglobulin or TCR genes is indicative of a clonal process. PCR testing has several advantages over Southern analysis, including increased sensitivity, smaller amounts of clinical sample with which to run the assay, and decreased time to perform the test.

Chromosomal translocations are common in lymphoproliferative disorders and therefore can provide useful markers of malignancy. Many oncogene translocations may contribute to the transformation process or cellular proliferation (Table 21-2).

The use of microarray technology has been used to define the gene expression profile of various lymphoid malignancies and to compare them to normal lymphoid populations. This 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. It also has identified novel genes that may be important for malignant transformation, which could increase our understanding of lymphomagenesis and potentially elucidate novel therapeutic targets.

Classification of non-Hodgkin lymphomas

The classification of lymphoproliferative disorders has evolved as a result of an increased understanding of the biology of these diseases. The current classification system used is the *World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues*, which was updated in 2008 (Table 21-3) (Swerdlow et al., 2008). The B- and T-cell neoplasms are separated into precursor (lymphoblastic) neoplasms and mature B- or T-cell neoplasms. There have been a number of updates and disease refinements in both the B-cell lymphoma and T-cell lymphoma sections of the WHO classification. Overall, ~90% of all non-Hodgkin lymphomas (NHLs) in Western countries are of mature B-cell origin, with DLBCL and FL being the most

Table 21-2 Phenotypic markers and chromosomal translocations in non-Hodgkin lymphomas.

NHL	sIg	CD5	CD10	CD20	Other	Cyclin D1	Cytogenetics	Oncogene	Function
CLL/SLL	Weak	+	-	Dim	CD23+ FMC-	-	No diagnostic abnormalities*	-	-
Follicular	++	-	+	+	-	-	t(14;18)	BCL2	Anti-apoptosis
Mantle cell	++	+	-	+	CD23- 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+/-	-	t(9;14)	-	-
Hairy cell leukemia	++	-	-	+	CD11c+, CD25+, CD103+	Weak	-	-	-
DLBCL	+	Rare	+/-	+	-	-	t(14;18), t(3;14), t(3;v) Rare t(8;X),	BCL2 BCL6	Anti-apoptosis Transcription factor Proliferation
PMBCL	+	-	-/+	+	CD30+/-	-	t(16;X)**	CIITA	MHC class II transactivator
Burkitt lymphoma	+	-	+	+	TdT-	-	t(8;14), t(2;8), t(8;22)	cMYC	Transcription factor
ALCL, ALK-positive	-	-	-	-	CD30+, CD2+/-, CD3-/+ EMA+	-	t(2;5)	ALK	Tyrosine kinase
ALCL, ALK-negative	-	-	-	-	CD30+, CD2+/-, CD3-/+ EMA	-	t(6;7)(p25.3;q32.3)	DUSP22	Phosphatase

* A number of prognostic cytogenetic abnormalities have been identified (see Chapter 22).

** A number of partner chromosomes described.

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; sIg = surface immunoglobulin; SLL = small lymphocytic lymphoma; TdT = terminal deoxynucleotidyl transferase.

common subtypes. In children, Hodgkin lymphoma (HL) is more predominant, and the aggressive NHLs of lymphoblastic lymphoma, Burkitt lymphoma (BL), and DLBCL are much more commonly encountered than indolent neoplasms. The incidence of NHL is lower among Asian populations, in whom T-/NK-cell neoplasms are more frequent.

In the updated WHO classification, there are a number of new designations in the category of DLBCL, with morphologic, molecular, and immunophenotypical subgroups as well as distinct disease entities recognized. DLBCL—not otherwise specified (DLBCL-NOS) includes all DLBCL cases that do not belong to specific subtypes or disease entities. It includes morphologic (eg, centroblastic and immunoblastic), molecular (ie, germinal center B-cell like [GCB] and activated B-cell like [ABC]), and immunohistochemical (eg, GCB vs. non-GCB) subgroupings (Table 21-3). Specific DLBCL disease subtypes include the new WHO designations primary central nervous system (CNS) DLBCL and EBV-positive DLBCL of the elderly. Additionally, there are a number of recognized lymphomas of large B-cells (eg, primary

mediastinal large B-cell lymphoma [PMBCL], intravascular lymphoma, lymphomatoid granulomatosis) (Table 21-3). Of note, borderline cases, the so-called gray-zone lymphomas (GZLs) also are distinguished, including an overlapping category between DLBCL and BL and an overlapping category between DLBCL (PMBCL) and classical Hodgkin lymphoma (cHL) (Table 21-3).

Few changes have been introduced to the indolent B-cell lymphoma classification. A variety of entities are considered small B-cell clonal lymphoproliferations that do not fall into the established categories of small B-cell lymphomas, including splenic B-cell lymphoma/leukemia, unclassifiable. In addition, a new category called primary cutaneous follicle center lymphoma, which typically presents as a localized lesion on the head and trunk (Table 21-3).

For the peripheral T-/NK-cell neoplasms, also collectively called peripheral T-cell lymphomas (PTCLs), the previously established categories of predominantly leukemic, predominantly nodal, and predominantly extranodal have been eliminated given that the subdivisions were often overlapping.

Table 21-3 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*
B-lymphoblastic leukemia/lymphoma NOS	T-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities	
Mature B-cell neoplasms	Mature T-cell neoplasms
<i>Aggressive lymphomas</i>	<i>Aggressive lymphomas</i>
Diffuse large B-cell lymphoma: variants, subgroups, and subtypes/entities	T-cell prolymphocytic leukemia
Diffuse large B-cell lymphoma, NOS	Aggressive NK-cell leukemia
Common morphologic variants: centroblastic, immunoblastic, anaplastic	Peripheral T-cell lymphoma, NOS
Rare morphologic variants	Angioimmunoblastic T-cell lymphoma
Molecular subgroups: germinal center B-cell like (GCB) and activated B-cell like (ABC)	Anaplastic large-cell lymphoma, ALK positive
Immunohistochemical subgroups: CD5 ⁺ DLBCL, GCB, and non-GCB	Anaplastic large-cell lymphoma, ALK negative
Diffuse large B-cell lymphoma subtypes	Extranodal NK/T-cell lymphoma, nasal type
T-cell/histiocyte-rich large B-cell lymphoma	Enteropathy-type T-cell lymphoma
Primary DLBCL of the CNS	Hepatosplenic T-cell lymphoma
Primary cutaneous DLBCL, leg type	Subcutaneous panniculitis-like T-cell lymphoma
EBV-positive DLBCL of the elderly	Adult T-cell leukemia/lymphoma
Other lymphomas of large B cells	Primary cutaneous $\gamma\delta$ T-cell lymphoma
Primary mediastinal large B-cell lymphoma	Primary cutaneous CD8+ aggressive epidermotropic T-cell
Intravascular large B-cell lymphoma	lymphoma
DLBCL associated with chronic inflammation	
Lymphomatoid granulomatosis	
ALK-positive large B-cell lymphoma	
Plasmablastic lymphoma	
Large B-cell lymphoma arising in HHV-8-associated multicentric Castleman disease	
Primary effusion lymphoma	
Borderline cases	
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma	
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	
Burkitt lymphoma	
Mantle cell lymphoma	
<i>Indolent lymphomas</i>	<i>Indolent lymphomas</i>
Follicular lymphoma	T-cell large granular lymphocytic leukemia [†]
Primary cutaneous follicle center lymphoma	Chronic lymphoproliferative disorders of NK cells
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT)	Mycosis fungoïdes
Nodal marginal zone lymphoma	Sézary syndrome
Splenic marginal zone lymphoma	Primary cutaneous CD30 ⁺ T-cell lymphoproliferative disorder
Splenic B-cell lymphoma/leukemia, unclassifiable	Primary cutaneous CD4 ⁺ small/medium T-cell lymphoma
Lymphoplasmacytic lymphoma	
Heavy chain disease	
Plasma cell neoplasms	
CLL/SLL	
B-cell prolymphocytic leukemia	
Hairy cell leukemia	

* All precursor neoplasms are considered aggressive.

[†] Course is usually indolent but in some cases is aggressive.

CLL = chronic lymphocytic leukemia; CNS = central nervous system; DLBCL = diffuse large B-cell lymphoma; HHV-8 = human herpesvirus 8; NK = natural killer; NOS = not otherwise specified; SLL = small lymphocytic lymphoma.

The classification system has been further refined with a number of important modifications, including recognition of several new distinct and provisional disease categories (Table 21-3). Anaplastic lymphoma kinase (ALK)-positive anaplastic large-cell lymphoma (ALCL) is now recognized as a distinct entity and ALK-negative ALCL is a provisional entity (see the section Systemic Anaplastic Large-Cell Lymphoma). The rare peripheral T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma (SCPTCL), is confined to cases with an $\alpha\beta$ phenotype. Several new primary cutaneous PTCL categories have been created because of differences in clinical behavior, including primary cutaneous $\gamma\delta$ T-cell lymphoma, which also includes the $\gamma\delta$ subtype of SCPTCL because of a similar aggressive course. Of note, one entity has been removed from this section of the WHO classification, blastic NK-cell lymphoma, and is listed in a new category called acute leukemias of ambiguous lineage.

For clinical purposes, the NHLs can be broadly separated into indolent or aggressive categories (Table 21-3). *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 usually 8–10 years but not uncommonly may exceed 15–20 years. 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 2011, there were 66,360 new cases of NHL, representing 4.2% of all cancer diagnoses. 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 21-1). Agricultural workers with cutaneous exposure to these agents have an approximately two- to sixfold increased incidence of NHL, possibly contributing to the relatively greater frequency of lymphoma in rural versus

urban populations. In children, NHL accounts for ~8% of all childhood cancers.

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 EBV (Table 21-1). More subtle, chronic immunoregulatory disorders, such as rheumatoid arthritis, Sjögren syndrome, and Hashimoto thyroiditis, also carry an increased risk of NHL. In children, the incidence of NHL is increased in several disorders that have in common immunodeficiency from primary immune disorders, including ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable or severe combined immunodeficiency, and X-linked lymphoproliferative disorder.

In general, there is no particular predilection for lymphoma among specific ethnic groups, although NHL is more frequent in Western than in Asian populations. Familial predisposition to NHL is rare; however, kindreds with high frequencies of NHL have been reported. In particular, CLL and Waldenström macroglobulinemia (WM) are seen more frequently in first-degree relatives.

Infection with the bacterium *Helicobacter pylori* is strongly associated with gastric MALT lymphoma. Interestingly (Table 21-1), patients with MALT limited to the stomach often achieve CR following successful therapy to eradicate *H. pylori*, indicating that the lymphoma remains dependent in part on continued antigenic drive. Recently, associations have been made between orbital infection by *Chlamydia psittaci* and orbital adnexal MALT lymphoma, infection with *Campylobacter jejuni* and immunoproliferative small intestinal disease, and *Borrelia burgdorferi* and cutaneous MALT lymphoma. These intriguing associations need to be firmly established by additional investigation, with variable responses observed with antimicrobial agents.

Certain viral infections have been linked with specific subtypes of NHL. EBV has a clear pathogenic role in endemic as well as some cases of sporadic BL and in many cases of HIV-related aggressive B-cell lymphoma. 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. It is also detected in 70%–80% of cases of angioimmunoblastic T-cell lymphoma (AITL); however, its role in disease pathogenesis is unknown. A new entity in the 2008 WHO classification has classified an EBV-associated DLBCL of the elderly. The γ -herpesvirus HHV-8 (Kaposi sarcoma-associated herpesvirus [KSHV]) was first described in Kaposi sarcoma but also has been associated with an unusual primary body cavity lymphoma (primary effusion lymphoma) 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 lymphoplasmacytic lymphoma.

Specific chromosomal translocations are associated strongly with individual subtypes of B-cell NHL (Table 21-2). 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 pathogenetic 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, the nucleophosmin promoter, and is found in a subset of Ki-1 (CD30)-positive 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).

Gene signatures in lymphoma

The diagnostic accuracy of lymphomas is now significantly improved; however, within any given lymphoma subtype, a diverse spectrum of clinical behavior reflects the underlying molecular genetic alterations inherent within tumor cells. The most notable studies of gene expression profiling in lymphoma have been in DLBCL. Prior landmark studies have established that morphologically similar DLBCL is composed of the ABC type with a signature reminiscent of an ABC and GCB type rich in GC markers. Importantly, the ABC type is associated with a more aggressive course. Studies also have established a unique gene signature for PMBCL, which has overlapping features of DLBCL and cHL.

The recent sequencing of the human genome facilitates more genomewide approaches using large-scale gene expression analyses that have been applied to simultaneously monitor the expression of thousands of genes from human tumor samples. Such studies have the potential to further refine the classification of heterogeneous disease sets, define molecular signatures of prognosis, 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; and bone marrow biopsy. CT or magnetic resonance imaging (MRI) of the brain and evaluation of the cerebrospinal fluid are indicated in patients with Burkitt or lymphoblastic lymphomas and also should be considered in patients with aggressive histology lymphoma involving high-risk sites, including the sinuses or testis. The Ann Arbor staging system, identifying patients as having stage I (localized) to stage IV (extensive) 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 symptoms, namely, fevers, drenching night sweats, or weight loss of 10% or more within 6 months of diagnosis (Table 21-4). Several limitations become apparent when the Ann Arbor classification is applied to NHL. Unlike HL, which has a contiguous pattern of lymphatic involvement, NHLs have a tendency to spread hematogenously and involve noncontiguous lymph node sites. In addition, the Ann Arbor staging system does not reflect the unique natural history of specific NHL subtypes or the consequences of lymphomatous involvement of certain extranodal disease sites. In addition, important factors reflecting tumor burden (eg, lactate dehydrogenase [LDH], number of nodal or extranodal sites involved, tumor bulk, β_2 -microglobulin, B-symptoms) and physiologic reserve of the patient (eg, age, performance status [PS]) are not included in this conventional staging system.

To more fully incorporate additional relevant prognostic features, more broadly relevant models have been developed in the most common NHLs, DLBCL and FL, and, more recently, MCL.

The most widely used clinical prognostic model to stratify patients with aggressive NHLs is the International Prognostic Index (IPI; Shipp et al., 1993). Institutions from around the world provided clinical and laboratory information on patients with aggressive large-cell lymphoma diagnosed by the Working Formulation, Kiel, and Rappaport classifications, and thus, immunophenotyping information was not available or incorporated into the model. On the basis of disease frequency, the most common subtype submitted for the development of the IPI would have been DLBCL. 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 independently associated with clinical outcome and often are referred to as APLES: (i) age >60 years, (ii) PS >2, (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

Stage	Definition [†]
I	Involvement of a single lymph node or of a single extranodal organ or site (IE)
II	Involvement of two or more lymph node regions on the same side of the diaphragm, or localized involvement of an extranodal site or organ (IIE) and one or more lymph node regions on the same side of the diaphragm
III	Involvement of lymph node regions on both sides of the diaphragm, which also may be accompanied by localized involvement of an extranodal organ or site (IIIE) or spleen (IIIS) or both (IIISE)
IV	Diffuse or disseminated involvement of one or more distant extranodal organs with or without associated lymph node involvement

*Fever 38 °C, night sweats, and weight loss 10% of body weight in the 6 months preceding admission are defined as systemic symptoms.

[†]The spleen is considered nodal.

Table 18-6 International Prognostic Index (IPI) and age-adjusted index for aggressive lymphoma patients treated with doxorubicin-containing combination chemotherapy.

additive score from 0-5 and has been widely adopted to estimate prognosis in patients with NHL and is useful in some of the other lymphoma subtypes (Table 21-5). Four prognostic risk categories were identified that had the following 5-year OS rates: low risk, 0 to 1 factor = 73%; low-intermediate risk, 2 factors = 51%; high-intermediate risk, 3 factors = 43%; and high risk, 4 to 5 factors = 26%. Of note, these survival estimates established before the use of rituximab diffuse large B-cell lymphoma.

An age-adjusted score has been developed for patients <60 years of age, where stage, PS, and elevated LDH, but not the number of extranodal sites, correlate with outcome (Table 21-5). These clinical IPI factors likely represent surrogate markers of the underlying biology of the lymphoma. Other serum and tumor markers, plus recent gene expression studies, hold promise to provide additional insights into pathogenesis and prognosis that in the future may have clinical utility to develop the treatment approach for individual

patients. 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 (Table 21-2). 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 21-6). This index often is remembered by No-LASH. The five clinical factors that are the strongest predictors of outcome in multivariate analysis were: (i) number of nodal sites of disease (>4), (ii) elevated LDH, (iii) age >60 years, (iv) stage III or IV disease, and (v) hemoglobin <12 g/L. 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 (>3 factors). The 10-year OS rates were 71% (low risk), 51% (intermediate risk), and 36% (high risk), respectively (Table 21-6). Similarly, an international prognostic index for MCL (the Mantle Cell Lymphoma International Prognostic

Table 21-5 The International Prognostic Index (IPI) in large cell lymphoma in the pre-rituximab era.

Risk group	Risk factors (no.)	Distribution of cases (%)	CR rate (%)	5-year OS (%)
<i>All ages*</i>				
Low (L)	0, 1	35	87	73
Low-intermediate (LI)	2	27	67	51
High-intermediate (HI)	3	22	55	43
High (H)	4, 5	16	44	26
<i>Age-adjusted index (<60)[†]</i>				
Low (L)	0	22	92	83
Low-intermediate (LI)	1	32	78	69
High-intermediate (HI)	2	32	57	46
High (H)	3	14	46	32

*IPI risk factors are age >60 years, abnormal LDH, PS ≥2, stage III or IV, and >1 extranodal sites.

[†]Age-adjusted IPI risk factors are age <60 years, normal LDH, PS ≥2, and stage III or IV.

CR = complete remission; LDH = lactate dehydrogenase; OS = overall survival; PS = performance status.

Shipp MA, Harrington DP, Anderson JR, et al. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med*. 1993;329:987-994.

Table 21-4 Ann Arbor staging system.*

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	27	53	36
IPI†				
Low	0-1	49	88	67
Low-intermediate	2	31	71	50
High-intermediate	3	15	57	28
High	4-5	5	44	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.

†IPI risk factors are age ≥60 years, abnormal LDH, PS ≥2, stage III or IV, and >1 extranodal sites.

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	≥70	—	≥1.50	≥5000

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.

Index [MIPI]) also has been developed, incorporating age, performance status (PS), LDH, and white blood cell (WBC) level (Table 21-7).

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, assessing 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 established as standard approaches and remain experimental (see the section DLBCL). The IPI is useful in comparing studies and also in the investigation of new prognostic factors to determine the independent effect on outcome.

The use of positron emission tomography (PET) scanning is proving increasingly useful both for staging and assessing response to lymphoma therapy. The International Harmonization Project in lymphoma recently outlined recommendations on the role of PET scans in staging and response assessment. PET scanning in the curative lymphomas, DLBCL and HL, is recommended because it will facilitate assessment of disease

Table 21-6 Comparison of the Follicular Lymphoma International Prognostic Index (FLIPI) and the International Prognostic Index (IPI) in follicular lymphoma.

Table 21-7 The Mantle Cell Lymphoma International Prognostic Index (MIPI).

extent and also provide a pretreatment comparison for response assessment (Table 21-8). Given that PET is not widely available, however, it is not absolutely required. Cases with residual abnormalities on CT scans but are PET-negative are considered to be in a CR(PET negative). Midtreatment PET scanning has been associated with prognosis, whereby cases that are positive are associated with a high relapse rate. Clinical trials are ongoing as to whether this information can be used to change therapy and improve outcome. It is recognized that 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 a remission by CT scan if high-dose chemotherapy and stem cell transplant (HDC/SCT) are under consideration.

Patient follow-up

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. Radiotherapy to the head and neck region leads to decreased salivation with dental caries and if the

Table 21-8 Recommended timing of PET/CT scans in lymphoma clinical trials.

Histology	Pretreatment	Midtreatment	Response assessment	Posttreatment surveillance
<i>Routinely FDG avid</i>				
DLBCL	Yes*	Clinical trial	Yes	No
HL	Yes*	Clinical trial	Yes	No
Follicular NHL	No†	Clinical trial	No†	No
MCL	No†	Clinical trial	No†	No
<i>Variably FDG avid</i>				
Other aggressive NHLs	No†	Clinical trial	No‡‡	No
Other indolent NHLs	No†	Clinical trial	No‡‡	No

*Recommended but not required before treatment.

†Recommended only if ORR/CR is a primary study endpoint.

‡Recommended only if PET is positive before treatment.

CR = complete remission; CT = computed tomography; DLBCL = diffuse large B-cell lymphoma;

FDG = fluorodeoxyglucose; HL = Hodgkin lymphoma; MCL = mantle cell lymphoma; NHL = non-Hodgkin lymphoma; ORR = overall response rate; PET = positron emission tomography.

thyroid was included in the radiation field, a large proportion of patients eventually may become hypothyroid and the thyroid-stimulating hormone (TSH) level should be monitored with each follow-up visit. Women who have had mantle radiation should receive a mammogram 10 years after radiation or at age 40 years. In younger women, MRI breast imaging also can be considered given reduced sensitivity of mammogram. Long-term survivors are at risk of second malignancies. Once primary therapy has been completed and remission 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 small 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 3–6 months indefinitely. There is no evidence that routine CT or PET imaging affects outcome in the surveillance of patients.

Indolent B-cell NHL

The indolent B-cell lymphomas include the cell type's show in Table 21-3, and the most commonly encountered subtype is FL, which accounts for 20%–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 will be discussed in a separate chapter.

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 and CD10. The staging evaluation reveals widespread lymphadenopathy, involving five nodal groups, with the largest node measuring just over 3 cm and 10% marrow involvement. 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 low tumor burden by *Groupe d'Etudes des Lymphomes Folliculaires (GELF)* criteria.

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 versus 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 both aggressive and indolent lymphomas. The FLIPI has been developed specifically for FL.

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 relatively good (median OS >10 years) and appears to be improving in the modern era (Fisher et al., 2005).

FLs are derived from GCB and are graded based on the number of centroblasts per high-power field: grade 1 (0–5), grade 2 (6–15), and grade 3 (>15). Grade 3 is further classified into Grade 3A (centrocytes present) and grade 3B (solid sheets of centroblasts). For purposes of classification

and clinical management, grade 1 and 2 can be considered one entity and the WHO classification uses the term “grade 1-2”. Grade 3 FL is relatively rare (<20% of all FLs), and the natural history of this entity is less clear. Most contemporary clinical trials will allow grade 3A to be included with grade 1-2 cases, whereas grade 3B typically is excluded. It is believed that grade 3B is best managed like DLBCL, but whether it is curable remains unknown. 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.

In the updated WHO classification, there are a number of identified variants of FL. These include primary intestinal FL, extranodal FLs, and pediatric FL. Primary cutaneous follicular center lymphoma, a provisional entity in the updated WHO classification, 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. It typically occurs as solitary or localized skin lesions on the scalp, forehead, or trunk, and only 15% present with multifocal lesions. The clinical course is usually very indolent.

Gene expression profiling has been explored in FL. In the largest study, molecular signatures divided patients into four quartiles with widely disparate median survival times (3.9, 10.8, 11.1, and 13.6 years; Dave et al., 2004). Interestingly, the signatures largely consisted of nonmalignant cells from the microenvironment. One signature was termed *immune response-1* and was associated with a more favorable prognosis and had high expression of genes expressed in T-cells. In contrast, *immune response-2* had high expression of genes expressed in monocytes or dendritic cells. Whether these signatures can be used to develop targeted therapies or are still relevant in rituximab-treated patients is unknown.

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, derived from single-institution databases. Older studies suggested a proportion of patients might be cured with an 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%-50%, suggesting that cure is possible in a proportion of patients with this approach (Wilder et al., 2001). 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 (Campbell et al., 2011). As a result, contemporary strategies tend to utilize an involved field approach. Studies evaluating chemotherapy plus radiation (combined modality therapy [CMT]) have demonstrated improved progression-free survival (PFS) without an obvious effect on OS (Seymour et al., 2003). Therefore, the CMT approach is likely best reserved for the rare patient who presents with bulky (node >5 cm) limited stage FL. Finally, an alternative management strategy for this patient population is watch and wait. 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 85% of patients were alive at 10 years (Advani et al., 2004). These results suggest that selected patients can be spared radiation treatment and enjoy excellent outcomes.

Approach to patients with advanced-stage follicular lymphoma

Patients with advanced stage FL generally 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 will undergo a number of different treatments, often separated by several years. Advanced-stage FL can be thought of as a chronic disease that requires long-term management, and the management is largely a matter of 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, the tumor burden, the patient age and comorbidities, and the 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 21-9). 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 FL can be considered for watch and wait or single-agent rituximab. Patients with asymptomatic, high-tumor burden FL 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 3-4 cm range). Patients with symptomatic, low-tumor burden are uncommon and care should be taken to look for alternative causes of the patient-reported symptoms. If no alternative explanations are uncovered, then initiation of treatment is reasonable. From a decision-making standpoint, patients with symptomatic, high-tumor burden FL are the most

Table 21-9 Algorithm for the approach to the newly diagnosed FL patient.

	Low tumor burden	High tumor burden
Symptoms absent	Watch and wait vs. single-agent rituximab	R-chemotherapy +/- MR vs. watch and wait
Symptoms present	Single-agent rituximab vs. R-chemotherapy	R-chemotherapy +/- MR

R = rituximab; MR = maintenance rituximab.

straightforward. They require treatment, although there is little consensus on which treatment is best. Following are recent data to support the various management strategies.

Management of asymptomatic, low-tumor burden follicular lymphoma

Asymptomatic patients may be candidates for a strategy of watch and wait. To determine whether watch and wait is an option, one should make an assessment of the tumor burden. The GELF criteria (Table 21-10) are the most commonly used criteria to assess tumor burden and to assess eligibility for clinical trials. The watch-and-wait strategy was first advocated at Stanford University when two retrospective studies suggested no detriment in patient outcome. Three randomized clinical trials later confirmed the Stanford observations. Low-tumor burden FL patients assigned to watch and wait 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. All of these studies, however, were conducted in the pre-rituximab era. To date, there are no studies comparing rituximab plus chemotherapy to watch and wait, and there is only one randomized clinical trial comparing single-agent rituximab to watch and wait in patients with previously untreated, asymptomatic, low-tumor burden FL (Ardeshta et al., 2010). Patients were assigned to watch and wait (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. With a median follow-up of 32 months, the proportion of patients progression free at 3 years was 33%, 60%, and 81% in Arms A, B, and C, respectively. The proportion of patients free of chemotherapy or radiation at 3 years was 48%, 80%, and 91% in Arms A, B, and C, respectively. There is no difference, however, in the OS at 3 years (95% in all Arms). 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

Table 21-10 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
- Hgb <10 g/dL, ANC <1.5 x 10⁹/L, Plts <100 x 10⁹/L
- 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'Etudes des Lymphomes Folliculaires*; Hgb = hemoglobin; LDH = lactate dehydrogenase; Plts = platelets.

population, but still relatively infrequent at 13% and 3%, respectively. Patients in all treatment arms adapted to their illness over time. The patients identified as “anxious” adapted more readily when assigned to rituximab treatments.

The interpretation of this study vary. It is reasonable to conclude that: (i) given no OS difference observed to date, watch and wait remains a reasonable standard for the asymptomatic, low-tumor burden FL population; (ii) some benefits are associated with immediate rituximab therapy, such as improved PFS and a longer time to first chemotherapy (these benefits should be discussed with patients); and (iii) a subset of patients (perhaps 15%) with particular difficulty adjusting to their diagnosis may experience a QOL benefit from single-agent rituximab.

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 (Kahl et al., 2011). After induction therapy with single-agent rituximab, patients with low-tumor burden FL were randomized to receive maintenance rituximab until treatment failure or to be periodically re-treated with rituximab (re-treated 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 3.5 times as much rituximab. On the basis of these results, a re-treatment strategy is preferred if opting for single-agent rituximab in this patient population.

Primary therapy of symptomatic, high-tumor burden follicular lymphoma

The addition of rituximab to conventional chemotherapy has improved outcomes in FL, including response rates, PFS, event-free survival (EFS), and OS. Table 21-11 summarizes major studies combining rituximab with chemotherapy.

Clearly, rituximab added to chemotherapy is a therapeutic advance in FL; however, the optimal chemotherapy backbone remains unsettled. Data from the U.S. Lymphocare project indicates the most commonly used regimens in the United States are R-CHOP (rituximab, cyclophosphamide, vincristine, prednisone) (60%), R-CVP (rituximab, cyclophosphamide, prednisone) (27%), and R-fludarabine-based (13%) (Friedberg et al., 2009). The first randomized comparison of these regimens was recently presented (Federico et al., 2012). Patients were assigned to receive R-CVP, R-CHOP, or R-FM (fludarabine, mitoxantrone). After a median follow-up of 25 months, the 3-year times to treatment failure (TTFs) were 47%, 57%, and 60%, respectively (*P* values only significant between R-CHOP/R-CVP and R-FM/R-CVP). No OS difference was noted between the three regimens. Patients treated with the fludarabine-containing regimens had a higher rate of neutropenia. This preliminary analysis shows that R-CVP is inferior to R-CHOP and R-FM for time to treatment failure, whereas R-CHOP and R-FM had similar efficacy. R-CHOP, however, appeared to have a better toxicity profile than R-FM. The author's conclusion was that R-CHOP offered the best risk–benefit profile of the three regimens.

These data were generated before the introduction of bendamustine as a therapeutic option. Bendamustine is a novel alkylating agent approved by the U.S. Food and Drug Administration (FDA) for the treatment of rituximab-refractory indolent lymphoma (Kahl et al., 2010). Results of a study comparing R-bendamustine to R-CHOP, as initial therapy for advanced FL, indolent lymphoma, and MCL were presented at the 2009 American Society of Hematology meeting and updated at a plenary session at the 2012 American Society of Clinical Oncology (ASCO) Meeting (Rummel et al., 2012). In this multicenter phase III study, 549 patients with high-tumor burden indolent NHL and MCL (median age 64 years) were randomized to receive bendamustine 90 mg/m² on day 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 overall response rates (ORR) were similar in the BR versus 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 after BR compared with R-CHOP (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 may be the preferred option for untreated FL. A confirmatory

Table 21-11 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	223	1.5 years	96%	88%	95%
	vs.	vs.		vs.	vs.	vs.
	CHOP	205		90%	70% (2-year DoR)	90% (2-year OS)
Marcus, et al. <i>J Clin Oncol</i> . 2008	R-CVP	162	4.5 years	81%	38 months	83%
	vs.	vs.		vs.	vs.	vs.
	CVP	159		57%	14 months (median DoR)	77% (4-year OS)
Herold, et al. <i>Ann Onc</i> . 2011	R-MCP	181	6 years	92%	57%	80%
	vs.	vs.		vs.	vs.	vs.
	MCP	177		75%	25% (6-year PFS)	65% (6-year OS)
Bachy, et al. <i>Ann Onc</i> . 2011	R-CHVP-IFN(6)	175	8 years	81%	44%	70%
	vs.	vs.		vs.	vs.	vs.
	CHVP-IFN(12)	183		72%	28% (8-year EFS)	79%* (8-year OS)

* *P* value not significant.

CVP = cyclophosphamide, vincristine, prednisone; DoR = duration of response; DFS = disease-free survival; EFS = event-free survival; MCP = mitoxantrone, chlorambucil, prednisone; R-CVP = rituximab, cyclophosphamide, vincristine, prednisone; R-MCP = rituximab, mitoxantrone, chlorambucil, prednisone.

randomized phase III trial (BRIGHT trial) was conducted in the United States and recently has completed accrual.

The question of whether to administer maintenance rituximab after frontline R-chemotherapy was addressed in the phase III PRIMA trial (Salles et al., 2010). 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. Induction treatment was selected by center; R-CHOP (75%), R-CVP (22%), or R-FCM (3%). Patients were randomized to either observation or 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 ($P < .0001$). 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, but given that maintenance rituximab generally is well tolerated, it has become a commonly utilized strategy in the United States. Regarding the duration of maintenance rituximab, available data suggest that 2 years is safe but data beyond 2 years is lacking.

Radiolabeled anti-CD20 antibodies (radioimmunotherapy [RIT]), which utilize both the sensitivity of NHL to radiation as well as anti-CD20 targeting, have been studied as consolidation in the frontline treatment of FL. A U.S. intergroup trial comparing R-CHOP to CHOP followed by I^{131} tositumomab consolidation in previously untreated FL was recently presented. After a median follow-up of 4.9 years, the 2-year PFS was 80% versus 76% ($P = .11$) and the 2-year OS was 93% versus 97% ($P = .08$) for the 526 evaluable patients randomized to either CHOP-tositumomab or R-CHOP, respectively. Thrombocytopenia was higher in the tositumomab group (46% vs. 6%, $P < .0001$), whereas febrile neutropenia was greater in the rituximab group (16% vs. 10%, $P = .05$). Given the lack of a PFS or OS benefit observed after RIT consolidation, presently, there is not a defined role for RIT in the initial management of FL.

Therapy for relapsed and refractory follicular lymphoma

Multiple options exist for the treatment of patients who have failed 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 high-risk/high-reward strategies, such as allogeneic stem cell transplantation (alloSCT), with many options in between.

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 ORR of 75% with a median PFS of 9.3 months (Kahl et al., 2010). The FDA-approved dose of single-agent bendamustine is 120 mg/m² given intravenously on days 1 and 2 of 21-day cycles. Approximately two-thirds of patients required dose modifications or delays, mainly due to cumulative myelosuppression. In addition, most practitioners prefer to administer bendamustine with rituximab. An expert panel has published guidelines on bendamustine dosing when combined with rituximab, and a recommend 90 mg/m² on days 1 and 2 repeated every 28 days (Cheson et al., 2010).

For patients who relapse after an alkylator-based therapy, fludarabine-based regimens can be used. They should be used with caution in heavily pretreated or elderly patients, however, due to immunosuppression. Patients are at increased risk of *Pneumocystis carinii* and reactivation of herpes zoster. Prophylaxis with trimethoprim/sulfamethoxazole and acyclovir should be considered, particularly in elderly patients. Furthermore, if ASCT is considered as a future treatment option, the number of cycles with fludarabine should be minimized to avoid stem cell toxicity.

For the rare patient with relapsed FL who did not receive rituximab containing chemotherapy as part of initial therapy, R-CHOP with maintenance rituximab has been proven to be superior to CHOP alone in a recently updated EORTC study. Overall, 465 patients were randomized to receive CHOP or R-CHOP with a second randomization to maintenance rituximab (375 mg/m² Q 3 months for 8 doses) versus observation in those patients achieving a response (van Oers et al., 2010). Similar to studies in the primary setting, R-CHOP was superior to CHOP with regards to PFS and OS. Furthermore, patients who received maintenance rituximab, regardless of whether patient's received CHOP or R-CHOP, had an improvement in PFS (median 3.7 years vs. 1 year, $P < .001$) The 5-year OS was 74% in the rituximab maintenance arm and 64% in the observation arm ($P = .07$).

RIT is also a viable option for patients with indolent B-cell NHL if the bone marrow is minimally involved and the disease is not bulky. 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 possibly as maintenance, more and more patients are becoming rituximab refractory. For patients who are still rituximab sensitive, it is a particularly attractive option for elderly patients who will not tolerate cytotoxic agents well.

Stem cell transplantation

HDC with autologous stem cell rescue and alloSCT are both useful strategies in the management of FL, particularly for

younger patients with high-risk features, such as a brief remission to previous 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 (van Besien et al., 2003). A lower 5-year recurrence rate with allogeneic transplantations was offset by a higher treatment-related mortality (TRM) compared with autologous transplantation, leading to similar 5-year survival rates of 51%-62%. To reduce the TRM of allogeneic SCT, most centers now favor a nonmyeloablative strategy 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 Center (FHCC) demonstrated a 3-year OS and PFS of 67% and 54%, respectively (Rezvani et al., 2008), whereas a series of 47 patients treated at the M.D. Anderson Cancer Center revealed a 5-year OS and PFS of 85% and 83%, respectively (Khouri et al., 2008). Closer inspection of the two cohorts reveal wide differences in the patient populations, with the FHCC patients more heavily pretreated and many with active disease at the time of SCT. These results highlight the importance of patient selection in generating outcome data.

There is one small, randomized clinical trial (the CUP trial), examining ASCT versus standard therapy in patients with relapsed follicular lymphoma (Schouten et al., 2003). The study, conducted in the prerituximab 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) (Rohatiner et al., 2007). A cohort of 121 patients, with a median follow-up of 13.5 years, were noted to have a plateau in the remission duration curve beginning around year 8. Nearly half the patients were still in remission at 10-15 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.

Marginal zone lymphomas

The WHO classification separates the marginal zone B-cell lymphomas (MZL) into extranodal MZL of MALT, 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; they 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 21-2); 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. Examples include gastric MALT (*H. pylori*), cutaneous MALT (*B. burgdorferi*), ocular adnexal MALT (*C. psittaci*), nodal MZL (hepatitis C), SMZL (hepatitis C), parotid MALT (Sjögren syndrome), and thyroid MALT (Hashimoto thyroiditis). There is significant geographic variation in the association with certain microbial pathogens. For example, the prevalence of *chlamydophila psittaci* in patients with ocular adnexal MALT appears to be 50%-80% in Italy, Austria, Germany, and Korea, whereas it is observed infrequently in Japan, China, and the United States.

MALT lymphomas

Extranodal MZLs or MALT lymphomas constitute ~70% of all MZLs. They occur in mucosal sites, predominantly gastric or intestinal, and some nonmucosal extranodal sites, including the lung, salivary gland, ocular adnexa, skin, and thyroid. These sites often are affected by chronic infection or inflammation, 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, they are typically indolent lymphomas, with 10-year OS rates of 90% in many series. MALT lymphomas can be characterized as either 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. The infection and associated inflammation are central to gastric MALT pathogenesis, and tumor cells often require the presence of *H. pylori* (antigen dependence) for growth and survival. 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%-80% of gastric MALT lymphomas durably regress following effective *H. pylori* antibiotic therapy (Nakamura et al., 2012). 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 up to 6 months to 1 year. Repeat assessment of *H. pylori* either 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%-30% of cases. In the series reported by Nakamura et al. (2012), only 3 out 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 (Goda et al., 2010). 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-based combinations, 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) (Kuo et al., 2012).

Nongastric MALT lymphomas usually have a very indolent course, including in the one-third of patients who present with stage IV disease (Thieblemont et al., 2000). 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%) (Zucca et al., 2003). 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 (Goda et al., 2010). Many patients can be managed with a watch-and-wait approach. Patients with advanced-stage disease typically can be managed using the same principles used for FL. Recurrences tend to occur in a site-specific fashion (ie, pulmonary MALT tends to recur in the lung), and so monitoring can be primarily (although not exclusively) site directed. An emerging story is the association of *Chlamydophila psittaci* and ocular adenexal MALT. Several regions of Europe have reported associations in >50% of cases. A recent European multicenter phase II study, testing the efficacy of doxycycline, detected evidence of *C. psittaci* in 39 out of 44 patients (Ferreri et al., 2012). Lymphoma regression was observed in 65% of the doxycycline-treated patients and the responses tended to be durable. Nonresponse was associated with failure to eradicate the *C. psittaci* organism.

Nodal MZL

Nodal MZL, previously known as monocytoid B-cell lymphoma, also arises from marginal zone B-cells. Whenever nodal MZL is diagnosed, a careful history and physical examination should be pursued for a possible coexisting extranodal MALT lymphoma component, which may be identified in up to one-third of cases. It more commonly presents with advanced-stage 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 is 60%-70%; however, the EFS is only 30%, which

likely reflects more commonly encountered advanced-stage disease. Management is similar to the approach recommended in follicular lymphoma.

In the recent updated WHO classification, a new category was introduced—pediatric nodal MZL, which has distinctive clinical and morphologic characteristics. 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

SMZLs are rare. The median age at diagnosis is 68 years, and it is more common in females. Patients usually present with symptomatic splenomegaly. Generalized lymphadenopathy is uncommon, but patients may have associated splenic hilar nodal or hepatic involvement. The bone marrow and blood typically are involved and villous lymphocytes may be seen. Diagnosis usually is based on spleen histology following splenectomy or after bone marrow examination. Clinically, it can be confused with CLL, MCL, FL, HCL, or WM. Unlike CLL and MCL, it is typically CD5 negative. 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. There is no reliable immunophenotypic method to distinguish SMZL from WM, so morphologic and clinical features must be used. Clinically, SMZL is more likely to have significant splenomegaly, whereas WM typically has a larger monoclonal protein (Arcaini et al., 2009). A prognostic model, using hemoglobin <12 g/dL, elevated LDH, and albumin <3.5 g/dL, has identified three distinctive risk groups (Arcaini et al., 2006). OS at 5 years was 88%, 73%, and 50% for patients with 0, 1, and 2-3 risk factors, respectively. Splenectomy is considered the optimal first-line therapy in symptomatic patients or in the case of cytopenias due to splenomegaly. Single-agent rituximab has been reported to be remarkably active, with an ORR of 100% in a series of 16 patients. Regimens active in other indolent lymphomas are appropriate for patients requiring systemic therapy. Some cases have been associated with hepatitis C infection, and responses have been reported with clearance of the virus with pegylated interferon and ribavirin (Hermine et al., 2002).

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 21-3). They also may express CD25 and CD38, but it is not a consistent finding. WM is found in a significant subset of patients with LPL and is defined as LPL with bone marrow involvement and an IgM monoclonal gammopathy. The disease affects predominantly older patients; however, a familial predisposition can occur in up to 20% of patients who present at a younger age. Symptoms may be due to tumor infiltration (marrow, spleen, liver, and lymph nodes), circulating IgM macroglobulin (hyperviscosity, cryoglobulinemia, or cold agglutinin hemolytic anemia), and tissue deposition of IgM or other proteins (neuropathy, glomerular disease, or amyloid) can occur. Coagulopathies can result from IgM binding to clotting factors, platelets, and fibrin.

When serum viscosity is significantly elevated due to the IgM paraprotein, patients may experience visual disturbances, headaches, dizziness, decreased level of consciousness, cardio-pulmonary symptoms, or a bleeding diathesis, which constitute the hyperviscosity syndrome. Symptomatic patients with hyperviscosity should be treated promptly with plasmapheresis to lower the circulating monoclonal protein, followed by prompt institution of chemotherapy to control the malignant proliferation and further paraprotein production.

The treatment approach to lymphoplasmacytic lymphoma is more like that of indolent lymphoma or CLL than for a plasma cell dyscrasia. Patients without symptoms or disease-related complications are best managed by close monitoring and without treatment. Factors indicating that treatment is warranted include a hemoglobin <10 g/dL, platelet count <100, bulky adenopathy, symptomatic splenomegaly, symptomatic hyperviscosity, progressive peripheral neuropathy, symptomatic amyloidosis, cryoglobulinemia, cold agglutinin disease, or evidence of transformation (Treon et al., 2009).

Treatment guidelines have been put forth from the International Workshop on Waldenström macroglobulinemia (Dimopoulos et al., 2009). The panel recommends rituximab-based therapies, but there is no consensus on which chemotherapy agents are preferred. Given disease rarity, there are no randomized controlled studies to support one first-line regimen over another. Reasonable options include R-CHOP, BR, R-dexamethasone-cyclophosphamide (DRC), R-CVP, and R-2-chloro-2'-deoxyadenosine (R2CdA). If single-agent rituximab is used, one must be aware of the risk for abrupt increases in IgM levels and worsening symptoms of hyperviscosity, particularly if the pretreatment IgM level is >5,000 mg/dL (Treon et al., 2004). The combination of bortezomib, dexamethasone, and rituximab was evaluated in untreated patients with WM and demonstrated an ORR of 96% (Treon et al., 2009). Peripheral neuropathy was problematic, however, with 61% of patients discontinuing

bortezomib prematurely. For relapsed disease, regimens not utilized in the previous lines of therapy can be considered. Other novel agents such as thalidomide, alemtuzumab, and everolimus have shown activity. In addition, autologous or allogeneic transplantation are considered in younger patients with relapsed, high-risk disease. As with other indolent lymphomas, ASCT has diminishing value when the disease is relatively chemo-resistant, suggesting a window of opportunity in which to apply this strategy (Kyriakou et al., 2010). Allogeneic SCT can produce highly durable remission but must be weighed against a 3-year nonrelapse mortality rate of 25%-30% (Kyriakou et al., 2010).

Hairy cell leukemia

The typical presentation of hairy cell leukemia (HCL) is that of a middle-age man (median age, 50-55 years) with pancytopenia, splenomegaly, hepatomegaly, cytopenic complications (eg, infections, bleeding), and an inaspirable bone marrow (dry tap). HCL is rare, representing 2% of all leukemias. Making the proper diagnosis is crucial because of its generally favorable prognosis, with a 10-year OS exceeding 90%, and its excellent treatment response to nucleoside analogs (Grever, 2010).

The disease is diagnosed by its typical peripheral blood morphology 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 21-2). Marrow biopsy demonstrates a mononuclear cell infiltrate with a "fried egg" appearance of a halo around the nuclei and increased reticulin and collagen fibrosis. Interestingly, a recent study was reported of whole-exome sequencing in HCL that revealed that all cases of HCL harbor a mutation in the BRAF V600E, suggesting that it may be important in disease pathogenesis and may prove to be useful diagnostically, because it is not demonstrable in any other B-cell lymphoma (Tiacci et al., 2011). In fact, a recent case report indicated an excellent response to vemurafenib, an inhibitor of mutated BRAF, in a patient with refractory HCL.

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, patients may be initially observed. HCL has a unique 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 and it also is available as a subcutaneous injection. In one large series of 233 patients with long-term follow-up, the ORR and

CR rate with either of these agents was 97% and 80%, respectively (Else et al., 2009). The median recurrence-free survival was 16 years, and many of the relapses were observed 5–15 years after treatment, highlighting the unique natural history of this disease. It currently is recommended that assessment of response should be determined 4–6 months following the end of treatment and if only a PR is attained then a second course can be given (Jones et al., 2012). Although there are no randomized trials evaluating rituximab in this setting, given the high density of CD20, the addition of rituximab can be considered if re-treatment is necessary.

HCL-variant is categorized separately in the WHO 2008 classification and despite its name, it is considered unlikely to be related to HCL. 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 as 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 good PR in two-thirds of patients. Case reports have shown CRs after rituximab.

Transformation to aggressive lymphoma in indolent lymphomas

Transformation is the development of aggressive NHL in patients with underlying indolent lymphomas. It most commonly occurs in follicular lymphoma but can occur in any of the indolent lymphomas. The British Columbia Cancer Agency recently reported on the incidence and outcome of 600 patients with FL who subsequently developed transformed lymphoma. Diagnoses were either 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 a 10- and 15-year risk of 30% and 45%, respectively. Overall, the median posttransformation survival time was 1.7 years, with superior outcomes observed in limited-stage patients. Similar results were observed in a series from St. Bartholomew's, where histologic transformation was observed in 28% of patients with FL by 10 years. There are no reliable clinical factor's to predict the future risk of transformation, thus, repeat biopsy should be performed in relapsed FL. Histologically, DLBCL is the most frequently observed subtype, but double-hit lymphomas and what previously was referred to as Burkitt-like lymphoma, also are observed. The treatment is directed at the aggressive lymphoma and depends on a variety of factors, including age, comorbidities, and extent of prior treatment for the FL.

Key points

- Advanced-stage indolent NHL is treatable but not curable with standard chemotherapy.
- Follicular NHL is the most common indolent NHL.
- IFRT for stage I and II indolent lymphoma will lead to long-term remission and potentially cure in a subset of patients and remains the standard of care in most cases.
- Patients with asymptomatic, advanced-stage indolent NHL may be followed without specific therapy to assess the pace of disease, or single-agent rituximab maybe used to delay the use of systemic chemotherapy.
- Chemoimmunotherapy is used in patient's with symptomatic disease or risk factors by the GELF criteria.

Aggressive B-cell lymphomas

The most prevalent of the aggressive lymphomas is DLBCL. Other histologies in this category include MCL, BL, lymphoblastic lymphoma, and most of the T- and NK-cell lymphomas (Table 18-4). These neoplasms are characterized by a more acute presentation and, although often curable (except for MCL), are associated with relatively short survival in the absence of therapy-induced remission. 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 CT imaging, the largest nodal mass was 6 cm in the retroperitoneal region, bone marrow biopsy shows involvement with a low-grade lymphoma. Laboratory studies show a normal complete blood count (CBC) and chemistries aside from an LDH elevated 1.5 times normal. His ECOG PS is 2. Immunophenotypic stains of the lymphoma cells reveal them to express CD19, CD20, κ-light chains, BCL2, and MUM1/IRF4. They are negative for CD10 and BCL6 expression.

Diffuse large B-cell lymphoma

DLBCL is composed of large B-cells with a diffuse growth pattern. The new WHO classification recognizes several sub-categories of DLBCL, including molecular subtypes (GBC and ABC; see later sections); pathologic subtypes, including T-cell-rich B-cell lymphoma; and defined disease entities, including PMBCL. Other than primary CNS lymphoma (PCNSL) treatment approaches are largely similar for the DLBCL subtypes.

DLBCL constitutes 25%–30% of all NHLs and can present with nodal or extranodal disease. Bone marrow involvement occurs in ~11%–27% of cases, but a proportion of these cases are discordant with the presence of a low-grade B-cell

lymphoma. In addition to the B-cell markers CD20 and CD19, the neoplastic cells may also express CD10 (30%-60%), BCL6 (60%-90%), and IRF4/MUM1 (35%-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 are recognized: GCB, which has a gene expression profile similar to germinal center B-cells (CD10⁺ and BCL6⁺); and ABC, which has a profile similar to activated peripheral B-cells (IRF4/MUM⁺) with a prominent NFκB gene signature.

Treatment of advanced-stage DLBCL

The backbone of treatment of all subtypes of DLBCL is anthracycline-based treatment with CHOP chemotherapy. With this approach, ~40% of patients are cured. The aggressive lymphoma German Non-Hodgkin Study Group (DSHNHL) evaluated whether the addition of etoposide (CHOEP) or shortening the cycle interval from 3 weeks to 2 weeks (CHOP-14) improved outcome. Although, CHOEP appeared to improve EFS in young patients (<60 years) and CHOP-14 improved EFS and OS for elderly patients (>60 years), this benefit appeared to be negated in future studies by the addition of rituximab, which has been labeled as the “great equalizer” (Pfreundschuh et al., 2006).

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. In 2001, *Groupe d'Etude des Lymphomes de l'Adulte* (GELA) presented preliminary results at the American Society of Hematology annual meeting plenary session of a landmark phase III clinical trial in which 399 patients 60-80 years of age with previously untreated advanced-stage CD20+ DLBCL were randomized to receive CHOP for eight cycles or R-CHOP on a standard 21-day schedule. An improvement in all endpoints, including CR rate, EFS, and OS, favoring R-CHOP over CHOP was demonstrated. With longer follow-up, the results held, and R-CHOP quickly became the standard of care for advanced-stage DLBCL around the world (Coiffier et al., 2002) (Table 21-12). More recently, the outcome of patients in this study followed for a median 10 years was reported demonstrating a 10-year PFS for R-CHOP treated patients of 35% (vs. 20% with CHOP alone) and 10-year OS of 43.5% (vs. 27.6%) (Table 21-12). A similar phase III study was carried out by the U.S. ECOG intergroup (E4494) study comparing six to eight 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

Table 21-12 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. (GELA/III)	202	R-CHOP × 8 vs ×	Age 60-80 y	57% vs. 38% (2 y)	70% vs. 57% (2 y)
	197	CHOP × 8	Stage II-IV	35% vs. 20% (10 y)	43.5% vs. 28% (10 y)
Pfreundschuh et al. (MInT/III)	413	R-CHOP-like* × 6 vs	Age 18-60 y	79% vs. 58% (3 y)	93% vs. 84% (3 y)
	410	CHOP like* × 6	aaIPI 0 or 1	74% vs. 56% (6 y)	90% vs. 80% (6 y)
Pfreundschuh et al. (RiCOVER-60/III) [†]	306	R-CHOP-14 × 6	Age 61-80 y	66.5% (3 y)	78% (3 y)
	304	R-CHOP-14 × 8	Stage I-IV	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. (ASCO 2011) (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. (ASCO 2012) (LNH03-6B/III)	296	R-CHOP-21 × 8	Age 60-80 y	60% vs. 56% (3 y)	72% vs. 69% (3 y)
	304	R-CHOP-14 × 6	aaIPI >1		
Recher et al. (LNH03-2B/III)	196	R-ACVBP	Age 18-59 y	87% vs. 73% (3 y)	92% vs. 89% (3 y)
	183	R-CHOP	aaIPI 1		
Wilson et al. (NCI/II)	72	DA-EPOCH-R	Age >18 y	79% (5 y)	80% (5 y)
			Stage II-IV		

Survival estimates shown for rituximab-containing regimens only and are rounded off where applicable to the nearest whole number.

*87% DLBCL; CHOP-like = CHOP-21 or CHOEP-21 in 92%; radiotherapy given to sites of bulk, extranodal disease (physician's discretion).

[†]80% DLBCL.

EFS = event free survival; G-CSF = granulocyte colony-stimulating factor; GELA = *Groupe d'Etude des Lymphomes de l'Adulte*;

PFS = progression-free survival; MInT = MabThera International Study Group; NCRI = British National Cancer Research Institute Study;

OS = overall survival; R = rituximab; RiCOVER-60 = rituximab with CHOP Over Age 60 Years; y = year.

difference detected, although there was a benefit in TTF for the R-CHOP arm. The analysis was confounded to some extent by the secondary randomization to maintenance versus 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 either CHOP or CHOEP with or without rituximab. The rituximab-containing regimens demonstrated an improvement in EFS and OS (Pfreundschuh et al., 2006) (Table 21-12). The Rituximab With CHOP Over Age 60 Years (RICOVER-60) trial by the same group evaluated CHOP-14 for six or eight cycles, with or without rituximab in elderly patients and also demonstrated a significant improvement in all endpoints with the rituximab combinations (Pfreundschuh et al., 2008). Of note, the latter study also established that six cycles of R-CHOP-14 was associated with the best outcome. Although, six versus eight cycles of R-CHOP-21 have not been compared, six should be preferred in most circumstances to minimize toxicities.

Two randomized studies (GELA LNH-03-6B and the British National Cancer Research Institute [NCRI]) compared R-CHOP-21 with R-CHOP-14, and there was no improvement of FFS or OS using the shortened cycle interval, thus confirming that R-CHOP-21 remains the standard (Table 21-12). Recently, a more dose-dense rituximab regimen is under evaluation with eight cycles of rituximab given with CHOP-14 × 6. Early results suggest that elderly male patients appear to particularly benefit from this dose-dense rituximab as they seem to have a faster rituximab clearance. However, this remains experimental and awaits a confirmatory phase III trial.

Treatment of limited-stage DLBCL

Approximately 25% of cases of DLBCL are limited stage, which typically includes patient's with stage I disease and nonbulky (<10 cm) stage II disease. Some groups or studies also will include patients with bulky stage I disease and exclude patients with B-symptoms. A large randomized SWOG trial (SWOG-8736) established that CMT was superior to CHOP alone for the treatment of localized (stage I(E), non-bulky stage II(E)) aggressive lymphoma (Miller et al., 1998). In this study, the 5-year PFS (77% vs. 65%, $P = .03$) and OS (82% vs. 72%, $P = .02$) for three

cycles of CHOP followed by IFRT was superior to that of eight 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 because of an excess of late relapses, which was offset by increased toxicity in the chemotherapy-alone arm. A stage-adjusted IPI has been proposed for limited-staged disease that includes stage II disease, age >60 years, PS >2, and elevated LDH as risk factors. The 5-year OS rates reported in the updated follow-up for patients with 0, 1 or 2, and 3 risk factors were 94%, 71%, and 50%, respectively.

The benefit of rituximab has not been specifically analyzed in a randomized controlled trial in localized DLBCL. The MInT study did include some patients with localized disease by nature of the inclusion criteria. The SWOG completed a phase II study evaluating three cycles of R-CHOP, with four 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, the majority of whom had DLBCL. Patients had to have at least one risk factor by the stage-modified IPI (Persky et al., 2008). The study population was similar to the SWOG study described earlier, enabling a historical comparison to determine the impact of the addition of rituximab to CMT. The 2-year PFS was superior in the R-CHOP patients (95% vs. 83%). The SWOG group recently evaluated the efficacy of CHOP for three cycles and IFRT followed by consolidation with Yttrium-90 ibritumomab tiuxetan (Zevalin) in limited-stage DLBCL. The 2-year PFS and OS were 92% and 95%, respectively, suggesting that this may be a promising approach.

With potential acute and more concerning, long-term side effects of radiotherapy, there has been interest in whether a subgroup of patients with limited-stage DLBCL can be selected to receive chemotherapy alone. In the prerituximab era, GELA compared four cycles of CHOP alone with four cycles of CHOP and radiotherapy in elderly patients (>60 years) with localized aggressive (83% DLBCL) without any aaIPI risk factors. With a median follow-up of 7 years, there were no difference in EFS ($P = .6$) or OS ($P = .5$), suggesting that select group of low-risk patients can avoid radiotherapy. Limited retrospective studies evaluating this approach in the rituximab era support that R-CHOP alone should be further explored in limited stage patients. Currently, chemotherapy alone and risk-adapted therapy remain experimental and the standard treatment approach in limited-stage DLBCL is CMT using R-CHOP and IFRT.

Strategies to improve cure rates in DLBCL

Although the outcome of DLBCL has improved with R-CHOP chemotherapy, ~50% of patients still fail primary therapy. There are several ongoing approaches to improve outcome in

DLBCL, including the use of a new combination chemotherapy backbone (eg, ACVBP, DA-EPOCH), enhanced anti-CD20 monoclonal antibody (eg, obinutuzumab [GA101], ofatumumab), maintenance therapy (eg, enzastaurin), PET-adapted strategies, and targeted or personalized therapy.

GELA recently reported the results of a phase III randomized controlled trial (LNH03-2B) comparing the dose-intensive regimen R-ACVBP (adriamycin, cyclophosphamide, videsine, bleomycin, and prednisone) with sequential consolidation (methotrexate, rituximab/etoposide/ifosfamide, cytarabine) with standard R-CHOP in patients with DLBCL 18-59 y and only one adverse prognostic factor by the aaIPI (Recher et al., 2011). The dose-intensive regimen was associated with a more favorable PFS and OS but with higher toxicity.

In North America, dose-adjusted (DA)-EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab) currently is under evaluation in the management of DLBCL. The premise behind the backbone DA-EPOCH arose from in vitro studies that supported that tumor cell kill could be enhanced by extended drug exposure. Dose adjustments are also made based on the neutrophil nadir. With promising results seen with DA-EPOCH in previously treated patients, a phase II study was performed at the NCI (National Cancer Institute) evaluating DA-EPOCH-R in newly diagnosed DLBCL, which also incorporated information regarding cell of origin. The 5-year PFS and OS was 79% and 80%, the latter reflecting the low salvage rates in treatment failures. Cases that were BCL6 positive and thus reflecting a GCB phenotype had a more favorable prognosis than those that were BCL6 negative, suggesting that DA-EPOCH-R maybe of particular benefit in the GCB subtype. Although generally well tolerated, DA-EPOCH-R is associated with greater toxicity than R-CHOP, with hospitalization for febrile neutropenia occurring in 19% of cycles, despite mandatory granulocyte colony-stimulating factor (G-CSF) support. A phase III trial comparing DA-EPOCHR to R-CHOP in newly diagnosed DLBCL, with biomarker analysis to evaluate outcomes in the ABC and GCB subtypes, is near completion.

New anti-CD20 antibodies also are being studied in a variety of B-cell lymphomas, including GA-101. In contrast to rituximab, obinutuzumab (GA-101) has enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) and increased direct cell death induction but low-complement dependent cytotoxicity (CDC). A phase III study comparing R-CHOP to G-CHOP has been initiated for the first-line treatment of advanced stage DLBCL.

Primary transplant in advanced-stage DLBCL

It has been proposed that patients in IPI poor-risk groups might benefit from a more aggressive treatment approach,

such as HDC and ASCT or alloSCT. A recent European phase III randomized trial in aggressive NHL patients tested eight cycles of CHOP versus two cycles of cyclophosphamide, epirubicin, vindesine, and prednisone followed by HDC and ASCT and found higher 5-year EFS for patients in the transplantation arm. There was an OS benefit for transplantation in a subgroup analysis confined to the high-intermediate IPI risk patients. The role of ASCT in frontline therapy for DLBCL, however, also remains unclear in the era of rituximab. The SWOG group recently reported the results at ASCO 2011 of a randomized phase III study investigating the benefit of HDC/ASCT in first remission in patients with advanced-stage diffuse large-cell NHL with an aaIPI 2 or 3. This study was initiated in 1997 and did allow patients with a T-cell phenotype although by disease frequency, the majority of patients had DLBCL. The initial induction regimen was five cycles of CHOP ($n = 215$); however, this was amended to R-CHOP ($n = 182$) in DLBCL patients in 2003 to align with the new standard of care. Patients in at least a PR after five cycles of R-CHOP were randomized to receive either three further cycles or autotransplant using total body irradiation (TBI) or BCNU (carmustine)-based regimens. In the intention to treat (ITT) analysis, the 2-year PFS favored transplant (69% vs. 56%, $P = .005$), however, there was no difference in the 2-year OS (74% vs. 71%, $P = .32$). Of note, 18% of patients in the standard arm subsequently underwent treatment with salvage therapy and ASCT at relapse. In exploratory analyses, there was no differential treatment interaction by phenotype (B- vs. T-cell) or induction regimen (CHOP vs. R-CHOP). Patients with high-risk aaIPI, however, had a superior PFS and OS (2-year 82% vs. 64%) in the transplant group. Given the lack of OS for the group as a whole, primary transplant is still considered experimental in the primary therapy setting even in high-risk subgroups.

Clinical and biologic prognostic factors in DLBCL *International Prognostic Index*

Taken together, approximately 50%-60% of patients diagnosed with DLBCL will be cured with rituximab-based chemotherapy; however, low- and high-risk groups can be further defined by clinical and biological factors. Limited studies support that the IPI is still prognostic in the rituximab treatment era (Table 21-13). It has been proposed that a revised IPI (R-IPI) may define new risk groups in rituximab-treated patients: very good risk (0 risk factors, 4-year PFS 90%); good risk (1, 2 risk factors, 4-year PFS 70%); and poor risk (>3 risk factors, 4-year PFS 50%). The DSHNHL group also evaluated the usefulness of the IPI in patients enrolled on prospective clinical trials, with a predominance of low-risk patients, and found that it did

Table 21-13 The IPI in DLBCL in the rituximab era.

Risk group	Risk factors (no.)*	Distribution of cases (%)		4 year PFS (%)		4-year OS (%)	
		BCCA <i>n</i> = 365	DSHNHL <i>n</i> = 1062	BCCA	DSHNHL†	BCCA	DSHNHL†
Low (L)	0, 1	28%	52%	85%	87%	82%	91%
Low-intermediate (LI)	2	27%	21%	80%	75%	81%	81%
High-intermediate (HI)	3	21%	17%	57%	59%	49%	65%
High (H)	4, 5	24%	10%	51%	56%	59%	59%

Estimates are rounded off.

*IPI risk factors are age ≥60 years, abnormal LDH, PS ≥2, stage III or IV, and >1 extranodal sites.

†3-year PFS and OS.

BCCA = British Columbia Cancer Agency; DLBCL = diffuse large B-cell lymphoma; DSHNHL = German High-Grade Non-Hodgkin Lymphoma Study Group; OS = overall survival; PFS = progression-free survival.

effectively separate patients into the previously established risk categories although the difference between the high-intermediate and high-risk groups was small (Ziepert et al., 2010) (Table 21-13).

Other clinical prognostic factors

Although the IPI is robust and relevant in the modern rituximab treatment era, it does not capture all prognostic information. For example, disease bulk (>10 cm) is important and is associated with increased local failure rates. The importance of disease bulk was highlighted in the MInT trial. The authors suggested that 10 cm was a reasonable margin to delineate patients with bulky disease in the rituximab era.

Particular sites of extranodal disease also are associated with prognosis. Concordant involvement of the bone marrow with DLBCL but not discordant involvement with a low-grade lymphoma is associated with an inferior outcome (Sehn et al., 2011). The patient described earlier has an IPI score of 3 (advanced stage, poor PS, elevated LDH), placing him in a high-intermediate risk group with an expected 5-year probability of survival with R-CHOP of 50%-60%, and the bone marrow involvement does not affect outcome because it is low-grade lymphoma. Testicular involvement with DLBCL also is associated with an aggressive course with a propensity for late relapses, CNS relapse (leptomeningeal and parenchymal), and recurrence in the contralateral testicle. Even patients with localized disease should be treated with 6 cycles of R-CHOP with radiotherapy to the contralateral testicle. Strong consideration also is given for CNS prophylaxis as outlined in the following section.

Biological prognostic factors

Although the IPI is easy to apply and remains valid in the current treatment era, it fails to capture underlying biological heterogeneity. As described, DLBCL can be divided

molecularly into the GCB and ABC subtypes, which also have a distinct signature from PMBCL as will be described. ABC DLBCL consistently has an inferior prognosis, independent of the IPI and relevant in the R-CHOP era. Furthermore, it is clear that prognosis also is affected by the tumor microenvironment in which a signature rich in extracellular matrix and histocytes (stromal 1) is associated with a favorable prognosis and a signature rich in angiogenesis markers (stromal 2) is associated with an aggressive course (Lenz et al., 2008).

Immunohistochemical (IHC) algorithms have been used in an attempt to capture the cell-of-origin phenotype using a methodology that can be applied routinely in clinical practice. Hans et al. (2004) first reported 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 been proposed that also have a lower sensitivity than gene expression profiling. These results, however, have been inconsistent as to whether the cell-of-origin (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 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.

MYC is translocated in ~5%-10% of DLBCLs, and early studies have suggested that it is associated with an aggressive course in the pre- and postrituximab treatment eras (Barrans

et al., 2010; Savage et al., 2009). In some cases, there is also a t(14;18) involving BCL2, the so-called double-hit lymphomas that can occur after a proceeding FL or de novo as the first manifestation of disease presentation. The combination of MYC driving cellular proliferation and BCL2 preventing apoptosis has proven to be very difficult to cure. Double-hit lymphomas can occur with DLBCL or more commonly, B-cell lymphoma, unclassifiable with features intermediate between BL and diffuse large B-cell lymphoma (BCL-U; also called GZL) (Johnson et al., 2009). The outcome is extremely poor in lymphomas with dual BCL2 and MYC translocation B-cell lymphomas and these patients are considered for investigational therapies. 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 in DLBCL patients treated with R-CHOP chemotherapy (Green et al., 2012; Johnson et al., 2012); in one study, this also was correlated with gene expression information (Johnson et al., 2012). MYC protein expression was found in approximately one-third of cases, far more than that captured by fluorescence in situ hybridization (FISH) analysis (11%) or high MYC mRNA expression, suggesting the multiple roads of MYC deregulation exist. Importantly, MYC protein expression correlated with a poor outcome (Johnson et al., 2012; 5-year OS validation cohort 39% vs. 70%, $p < .001$; Green et al., 2012; 3-year 43% vs. 86%, $p < 0.001$), but only in the majority of cases with concurrent BCL2 expression. Furthermore, cases with a MYC translocation in the absence of BCL2 had a favorable outcome. The significance of this double-hit score is independent of the IPI and patients with a high IPI and dual-protein expression have an extremely aggressive course with a median PFS of only 6 months (Green et al., 2012). Further studies are needed to determine whether these patients should be treated with more dose-intensive regimens or agents targeted against MYC or BCL2.

The management of relapsed and refractory DLBCL

Given the implications of recurrent DLBCL, efforts should be undertaken to obtain a diagnostic biopsy unless there is unequivocal progression on CT imaging. A positive PET scan with stable disease should never be equated with disease recurrence given the potential for false positives. In addition, some patient's may relapse with indolent lymphoma, which would be managed very differently. Following confirmation of recurrence, patients should undergo full restaging investigations. If the patient does not have significant comorbidities and is <65 years (<70 in very select circumstances), second-line (salvage) combination chemotherapy regimen should be given such as ICE (ifosfamide, carboplatin, etoposide), DHAP (dexamethasone, AraC, cisplatin), or GDP

(gemcitabine, dexamethaxone, cisplatin) 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 PARMA study. Patients who relapsed with aggressive lymphoma (excluding CNS or bone marrow involvement) following an initial CR to primary therapy, received two cycles of DHAP salvage chemotherapy. If chemosensitivity (ie, a PR or CR to salvage chemotherapy) was demonstrated, patients were then randomized to receive either further chemotherapy with DHAP or HDC with BEAC (carmustine, etoposide, cytarabine, and cyclophosphamide) and ASCT. The transplant arm resulted in an improvement in both the 5-year EFS (46% vs. 12%, $P = .001$) and OS (53% vs. 32%, $P = .038$). The optimal salvage therapy recently has been investigated in two phase III 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 R-DHAP or R-ICE for three cycles followed by HDC (with BEAM)/ASCT if a response was demonstrated. There was also a second randomization following transplant to either rituximab or observation to evaluate the role of maintenance therapy (Gisselbrecht et al., 2010). 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 either EFS or OS and maintenance rituximab did not affect outcome. Importantly, patients who previously had received rituximab with their primary therapy had an inferior response rate (51% vs. 83%, $p < .001$) and 3-year EFS (21% vs. 47%), suggesting that this represents 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%, $P = 0.018$), 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. These results suggest selective drug sensitivity in molecular DLBCL subtypes but await confirmation in other data sets. A second phase III trial was presented at the ASH meeting in 2012 by the NCIC (National Cancer Institute of Canada) comparing DHAP to the outpatient salvage regimen GDP (gemcitabine, dexamethasone, cisplatin) in aggressive lymphomas using a noninferiority design. In 2005, the protocol was amended for aggressive B-cell lymphomas to include rituximab with each salvage regimen. The ORR, EFS, and OS was similar between the treatment arms but (R)-GDP was associated with less grade 3 or 4 toxicity ($P = .0003$), including, febrile neutropenia (9% vs. 23%, $p < .0001$) and patients had superior QOL scores.

There is very little information regarding the effectiveness of salvage therapy in patients with refractory disease, which typically is defined as either progressive disease (PD) on primary therapy or relapse within 3 months. In the CORAL study, only 11% of patients had PD on primary therapy. Limited studies suggest that these cases are rarely responsive to salvage therapy and most often are unable to undergo HDC/ASCT. Further patients with chemo-resistance to second-line therapy also have a very poor prognosis and these high-risk groups should be considered for investigational therapies.

The management of non-transplant-eligible patients with relapsed or refractory DLBCL, including novel therapies

Many patients are not eligible for curative intent treatment with salvage chemotherapy and HDC/ASCT due to advanced age or comorbidities. Given that the goal of treatment in this setting is typically disease control and not cure, single-agent chemotherapy often is used because it is less toxic than combination regimens. Use of the salvage regimens outlined previously in this typically older group of patient's usually is quite toxic, and it is unknown whether these regimens prolong OS. The exception is select cases with late relapses or low-secondary IPI risk score, which rarely may be cured with a salvage combination regimen. Gemcitabine and etoposide have been used in the palliative setting in addition to oral low-dose chemotherapy (metronomic) with good tolerance and modest response rates. Rituximab often is added given synergy with chemotherapy and good tolerability, although there is no firm data showing improved outcome. Palliative radiotherapy can be effective for localized, symptomatic disease. This is also a population in which entry into a clinical trial may be appropriate if the patient's PS is still good.

Primary mediastinal (thymic) large B-cell lymphoma

PMBCL was recognized as specific entity in the WHO classification based on unique clinicopathologic. Patients are typically females with a median age of 35 years who present with a bulky anterior mediastinal disease that can be locally invasive into the lung and chest wall occasionally with symptoms of superior vena cava syndrome. Distant spread, including bone marrow involvement, is uncommon at diagnosis. At relapse, involvement of unusual extranodal sites can occur in PMBCL, including the kidneys, adrenals, ovaries, liver, spleen, and CNS.

Histologically, sclerosis is typically present, and phenotypically, the cells may lack surface immunoglobulin expression but express B-cell markers, such as CD19 and CD20. CD30 is present in 80% of cases; however, it is usually weak and heterogeneous. Interestingly, recent 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 GZL with overlapping features of both malignancies is now defined in the WHO (see "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL"), further highlighting the biological continuum between these diseases.

Recently a novel recurrent translocation involving *CIITA* (MHC class II transactivator) was found to be recurrent in PMBCL, occurring in 38% of patients and also found in 15% of cHL (Steidl et al., 2011) (Table 21-2). Cases with these chromosomal breaks had an inferior disease-specific survival. Prior studies also found reduced expression of MHC class II genes, and this also is linked to an inferior outcome.

The outcome of patients with PMBCL is favorable even in the pre-rituximab treatment era (5-year OS, 70%; Savage et al., 2006), although patients have primary refractory disease and very low cure rates. Some retrospective studies have suggested an improvement in outcome with more dose-intensive regimens, such as MACOP-B (Zinzani et al., 2002); however, no randomized studies are available to definitively answer this question. Furthermore, with the routine use of rituximab, it is unknown whether this may eliminate any potential benefit of dose-intensive regimens. The MiNT study included 87 patients with PMBCL, which confirmed an improved CR(u) rate, a reduction of PD, and an improvement in 3-year EFS (78% vs. 52%, $P = .001$) with the addition of rituximab to CHOP-like chemotherapy, but the OS was similar (89% vs. 78%, 0.158) (Rieger et al., 2011). Similar outcomes using R-CHOP in PMBCL were reported in two other retrospective studies. DA-EPOCH-R also has been explored in a phase II study and the updated survival of 40 patients treated on this study was reported at Lugano in 2011 with encouraging results with a median follow-up of 47 months (EFS 95% and OS 100%) (Dunleavy et al., 2011). R-CHOP is considered the standard therapy in PMBCL; however, if these results are reproduced in other series, this would support DA-EPOCH as a reasonable alternative, particularly given that the vast majority of patients avoid radiotherapy.

Radiotherapy often administered to the mediastinum; however, it is unknown whether this is mandatory in all patients, particularly given the potential for long-term secondary effects in this relatively young patient population. Studies are ongoing investigating whether PET scanning can be used to select patients who may benefit from consolidative

radiotherapy and conversely spared if they are in a CR. Radiotherapy is not routine with DA-EPOCH-R, supporting the notion that some patients can be cured with chemotherapy alone. The international extranodal study group (IELSG) has initiated a randomized trial, whereby patients who are PET negative after a rituximab-containing regimen are randomized to observation versus radiotherapy.

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL

In the updated WHO 2008, a new category was defined that shows 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 may have supraclavicular lymph node involvement. A broad spectrum of cytological appearance can occur within the same tumor. The immunophenotype often is transitional between PMBCL and cHL (see Chapter 20) 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 GZL.

Given disease rarity, there is little information regarding clinical outcome; however, small series would suggest that this type of GZL tends to have a more aggressive course. Patients with GZL treated with DA-EPOCH-R appear to have an inferior outcome (EFS 45%, OS 75%) and often require radiotherapy (37%) (Dunleavy et al., 2011).

Primary CNS lymphoma

PCNSL can occur in the brain parenchyma, spinal cord, eye (ocular), cranial nerves, or meninges. Of note, although 95% cases of PCNSL are DLBCLs, rare cases of PTCL, low-grade lymphoma, and BL also have been reported. In addition to B-cell markers, CD10 expression is observed in only 10%-20% but BCL6 expression is common (60%-80%). PCNSLs are rare and may occur in immunocompetent patients or in association with immunosuppression related to HIV infection or organ and marrow transplantation. With the introduction of highly active retroviral therapy (HAART), 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%-20% of patients and may be the sole site of disease at presentation

(intraocular lymphoma). Concurrent leptomeningeal disease is found in 16% through CSF analysis but occurs as the sole site in <5%. B symptoms are extremely uncommon and should raise suspicions of systemic involvement (reviewed by Ferreri, 2012).

Stereotactic-guided biopsy is the optimal method to diagnosis CNS lymphoma and 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 CT imaging, bone marrow aspirate and biopsy, and, in men, testicular ultrasound as 4%-12% of patients can have extraneural 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 >60; 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 a 2-year OS rate 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. CHOP has poor CNS penetration and should not be used in PCNSL. The exception is intravascular large B-cell lymphoma with CNS involvement as 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) (3-8 g/m²) is incorporated into first-line regimens. With this approach, the 5-year OS is approximately 30%-40%. Some studies have added other CNS-penetrant chemotherapy drugs, such as cytarabine (ara-C). Rituximab has poor penetration across the blood-brain barrier but is currently being tested in clinical trials given synergy with chemotherapy. In younger patients, the combination of whole-brain radiation and HD-MTX often is used. A phase III trial randomizing younger patients in a CR following HD-MTX to either WBRT (45 Gy) versus observation demonstrated an improvement in median PFS (18 months vs. 12 months) but OS was similar (reviewed Ferreri, 2012). For older patients >60 years, the risk of neurotoxicity is considerable and manifests as dementia, ataxia, and incontinence, with a median time to onset of approximately 1 year. With 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, temozolamide, and rituximab with consolidative HDC using ara-C and etoposide without WBRT and the 3-year PFS and OS were 50% and 67%, respectively. Studies are also investigating lower doses of radiation in patients in a CR after chemotherapy. If chemotherapy is contraindicated because of age or comorbidities, WBRT 40–50Gy is recommended (Ferreri et al., 2012).

The outcome of primary intraocular lymphoma is similar to primary CNS lymphoma and the optimal therapy is unknown. Intravitreal MTX appears to be highly active and reduces the rate of local relapse but does not affect OS. In addition, limited studies also support that intravitreal rituximab is safe and effective and may limit the need for multiple MTX injections with concomitant toxicity. Approximately 60%–80% of patients with primary intraocular lymphoma can relapse in the brain parenchyma. Thus, regardless of the local therapy used, HD-MTX often is incorporated and it also has been shown to have good penetration into the eye.

HDC/ASCT has been evaluated in the upfront and salvage setting. Several small phase II studies have evaluated upfront transplant with cure rates ranging from 40% to 77% using a variety of lead-in chemotherapy and HDC regimens. There are two ongoing randomized trials comparing WBRT and HDC/ASCT. 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%. Currently, it should be considered experimental but may be appropriate in select young patients in the relapsed setting. Temozolamide either alone or in combination with rituximab has shown an ORR of 26% and 53%, respectively, in relapsed and refractory patients. No evidence suggests that intrathecal chemotherapy improves outcome if HD-MTX is being used; however, the CSF should be reanalyzed on treatment to ensure clearance of the malignant cells.

Secondary CNS lymphoma

The rate of secondary involvement of CNS in aggressive lymphoma varies by histology, occurring in up to 30% of BL (see “Burkitt’s lymphoma”) and lymphoblastic lymphoma. 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 prolong survival. Secondary CNS lymphoma also is seen in DLBCL occurring in the brain parenchyma, leptomeningeal compartment, or both, as an isolated event, or with systemic relapse. The overall risk of CNS relapse and progression in DLBCL is only ~5% but can be up to 25%–30% in specific high-risk subgroups. A number of extranodal sites have been associated with a high risk of CNS relapse, including testis, breast, kidney, sinuses, and bone

marrow (concordant). A Norwegian study proposed a risk model identifying five risk factors (age >60, elevated LDH, low albumin, >1 extranodal sites, retroperitoneal involvement) and supported that those with more than three factors, have a risk of CNS relapse of >25%. The National Comprehensive Cancer Network (NCCN) guidelines also identify patients as high risk for CNS relapse based on similar factors (paranasal, parameningeal, testis, bone marrow, and >2 extranodal sites).

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. The exception is sinus involvement, a site that does appear to benefit from CNS prophylaxis with intrathecal chemotherapy. 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) with a median survival from CNS relapse of 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 (Villa et al., 2009). 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. It is notable that the predominant pattern of CNS relapse in these diseases is leptomeningeal, which may be why a benefit is more difficult to demonstrate in DLBCL where parenchymal relapses are not uncommon, particularly in R-CHOP treated patients. In the prerituximab era, there is some evidence to support that use of systemic chemotherapy in DLBCL that penetrates the CNS may reduce the CNS relapse rate. Use of the intensive regimen ACVBP, which includes a consolidation phase incorporating CNS penetrant agents (methotrexate, etoposide, ifosfamide, cytarabine), was associated with a reduced risk of CNS relapse compared with CHOP. The CNS relapse rate using R-ACVBP is unknown, but presumably it would be reduced further. Prophylactic use of HD-MTX (3.0–3.5 g/m²) with R-CHOP was evaluated retrospectively in 65 patients with high-risk DLBCL (elevated LDH, involvement of >1 extranodal sites, 4–5 Hollender criteria, high-risk location: bone marrow, testes, epidural, liver, adrenal, renal, orbit) and reported a low rate of CNS relapse (3%) (Abramson et al., 2010). Use of HD-MTX,

however, is limited in elderly patients, particularly with poor renal function.

Despite the limitations and lack of evidence-based data to direct treatment, patient's considered high risk either by the extranodal site involved or by the Hollender risk model, should be evaluated for occult CSF involvement using cytology. Flow cytometry also has been shown to be a more sensitive tool for the detection of CNS involvement and should be employed where possible to rule out CNS disease at the time of diagnosis. Those with positive findings should undergo further staging with a MRI and be treated aggressively for CNS disease. Cases negative by CSF can be considered for prophylactic strategies and where possible evaluated in a prospective clinical trial. A management algorithm has been proposed in a recent comprehensive review (Siegal and Goldschmidt, 2012).

B-cell lymphoma unclassifiable with features between DLBCL and BL

B-cell lymphomas with features between intermediate between DLBCL and BL have morphologic and genetic features of both DLBCL and BL and typically a very aggressive course. Morphologically it appears intermediate between DLBCL and BL with cells usually smaller than typical DLBCL but larger than typical BL with a high proliferation rate, starry-sky appearance, and an immunophenotype consistent with BL. Other cases may be morphologically similar to BL but have an atypical immunophenotype or genetics. So-called double-hit lymphomas with dual translocation of cMYC and BCL2 are included in this category unless they are morphologically identical to DLBCL. Unlike BL, the MYC partner chromosome is often non-Ig. Overall, the prognosis is poor, and for the double-hit lymphomas, the risk of secondary CNS involvement is high and they rarely are cured with standard therapy (Johnson et al., 2009). Dose-intensive therapies, including CNS-penetrant drugs, are under investigation.

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, as depicted by nearly 100% of cells being 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 21-2). 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/lymphoma. Recent gene expression profiling studies show that BL has a distinct molecular signature distinguishing it from DLBCL (Dave et al., 2006).

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. Most endemic and some sporadic cases show evidence of EBV infection and presence of the EBV genome.

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 40% of patients; as a result, HD-MTX and intrathecal chemoprophylaxis are integrated into the therapy for 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 alkalinization, hydration, allopurinol with close monitoring of laboratory studies, including electrolytes and renal function. Early dialysis is indicated at the first signs of decreasing renal function, hyperkalemia, or hyperphosphatemia. Recently, 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. The most commonly used approaches today include intensive short-duration chemotherapy, ALL-type chemotherapy, and intensive chemotherapy with or without followed by high-dose chemotherapy and ASCT. Given disease rarity, there are no randomized controlled treatment trials in adults comparing these approaches. Magrath et al. (1996) 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, vinristine, doxorubicin, and methotrexate (CODOX-M) only, and high-risk patients received CODOX-M alternating with ifosfamide, etoposide, and cytarabine (IVAC) for a total of four cycles (ie, two 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 two cycles. Approximately half of the patients were adults, and the 2-year EFS for all patients was 92%. Two other phase II studies have used the Magrath regimen with minor modifications. In a United Kingdom study, adult (age range, 16–60 years; median, 26.5 years), non-HIV patients were treated with dose-modified CODOX-M (3 g/m²) for three cycles if determined to be low risk (ie, normal LDH, PS of 0 or 1, Ann Arbor stage I or II, and no tumor mass >10 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%. At the Dana-Farber Cancer Institute, an older population (median age, 47 years) of patients was treated with a modified Magrath regimen, and the reported 2-year EFS was 71% with a modified Magrath regimen. Other therapeutic approaches have included the hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (HyperCVAD)/methotrexate-cytarabine regimen and ALL-type regimens. Of note, there is no role for radiotherapy in the treatment of BL, even for localized disease. It also is unknown whether consolidative ASCT improves outcome in BL.

Given the limited data in older patients, the results from 12 large treatment series (10 prospective and 2 retrospective) were combined to better determine outcome in patients with BL in patients >40 years of age (Friedberg et al., 2005). In total, 470 patients were identified, 183 of whom were >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. Patients in the older age-group who underwent ASCT appeared to have a more favorable prognosis (2-year OS 62%), but this was a small group, and selection bias may have been introduced.

A few studies have been reported evaluating the impact of rituximab to intensive therapy. The addition of rituximab to CODOX-M/IVAC was evaluated in 40 patients with BL and compared with CODOX-M/IVAC alone. There was a reduction of relapses ($P = .01$) but only a trend to an improved outcome was observed for 3-year PFS (74% vs. 61%) and 3-year OS (77% vs. 66%) (Barnes et al., 2011). The outcome of 17 patients with BL (median age 27 years; HIV positive $n = 4$) treated with DA-EPOCH-R demonstrated an OS of 100% and EFS of 92% and may be an option for older patients who cannot tolerate more dose-intensive regimens, although caution is warranted as the frequency of grade 4 neutropenia was 47% (febrile neutropenia 16%). Interestingly, there was no reported tumor lysis syndrome, which may reflect the infusional chemotherapy used.

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.

HIV-associated lymphomas

HIV-associated lymphomas are typically DLBCL or BL. Approximately two-thirds of cases are EBV associated, and many carry a *cMYC* oncogene translocation. CNS involvement is frequent. The entity of primary effusion lymphoma (body cavity lymphoma) usually presents in HIV-positive patients as ascites or a pleural effusion but may involve soft tissue or visceral masses. It is pathogenetically associated with HHV-8 (KSHV) and generally carries a poor prognosis.

The therapy for HIV-associated lymphomas has used both full-dose and dose-modified combination chemotherapy regimens, usually with G-CSF support and can lead to modest cure rates. Typically highly active antiretroviral therapy (HAART) is given concurrently with chemotherapy and in communication with the HIV specialist to avoid antiretrovirals that can exacerbate chemotherapy toxicity,

but this approach has been recently challenged. Several registries have reported a significant decline in the incidence of HIV-associated lymphoma since the introduction of HAART.

The 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 II 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 in the HIV-negative setting. Concurrent administration of G-CSF is advised given the high rate of infection in this population. DA-EPOCH has been tested in HIV-aggressive lymphoma, the majority of which had DLBCL but with suspension of HAART to avoid any 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 either concurrently or sequentially, and the 2-year OS rate was 63% and 66%, respectively. HAART 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 (Sparano et al., 2010). More recently, the NCI piloted a second-generation regimen short course SC-EPOCH-RR (dose-dense rituximab), with G-CSF support, in HIV-positive DLBCL patients in the hope of improving efficacy but reducing toxicity. Dose-dense rituximab was intended to enhance the chemotherapy and minimize the number of treatment cycles and HAART was suspended during treatment. A PET scan was performed after two cycles: if negative, only one further 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 either 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%, $P = 0.01$) regardless of the regimen used. It remains unclear whether HAART is mandatory during chemotherapy; however, with the possible exception of the SC-EPOCH-RR, studies support continuing HAART in patients treated

with a variety of regimens, including Hyper-CVAD, CDE (infusional cyclophosphamide, doxorubicin, etoposide), EPOCH-R, and R-CHOP, particularly because newer antiretrovirals have fewer drug interactions than in the past. Use of Zidovudine is avoided because of increased risk of myelosuppression and the potential for deleterious drug interactions.

Many of these trials included both BL and DLBCL. The Spanish group PETHEMA reported the outcomes of HIV-positive BL treated with an intensive ALL-like regimen and HAART treatment and found that the outcome indiscernible from HIV-negative patients (2-year DFS 87% vs. 92%; 2-year OS 73% vs. 82%). Other studies have investigated Hyper-CVAD and modified CODOX-M/IVAC and DA-EPOCH and have reported encouraging results. The benefit of rituximab in this population remains unclear. The CNS relapse risk is high, and staging should include CNS evaluation at diagnosis and appropriate CNS prophylaxis. Taken together, the published data support that HIV-positive BL should be treated with similar regimens to HIV negative HL except with CD4 counts <50 given the high TRM in this population.

Posttransplant lymphoproliferative disorders

Post-transplant lymphoproliferative disorders (PTLDs) occur as a consequence of immunosuppression in recipients of solid organ, bone marrow, or stem cell allograft. The risk is higher in solid organ transplants that warrant a higher degree of immunosuppression (10%-25% in heart and lung transplant) compared with those that require a lower immune suppression dosing (1%-5% kidney and transplant). More than 90% of PTLDs in solid organ recipients are of host origin; however, in contrast, most PTLDs in bone marrow allograft recipients are of donor origin. PTLDs are composed of a spectrum of disorders, ranging from EBV-positive infectious mononucleosis (early lesions) to polymorphic PTLD, which most often are clonal to full-blown monomorphic PTLD that can be either EBV positive (common) or EBV negative and are further subdivided into B-cell lymphomas (common) and T-cell lymphomas (rare), and are indistinguishable from their counterparts in immuno-competent 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 is late onset (median time from transplant to PTLD of 50-60 months vs. 6 months in EBV positive), has 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 minority of patients will respond to a reduction in intensity of immunosuppressive drugs, most require additional systemic therapy particularly for monomorphic or late PTLDs. Furthermore, the median time to response is approximately 1 month, and there is an inherent concern of graft failure. Tolerance to chemotherapy is poor in PTCL patients with TRM 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 II 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% (Choquet et al., 2007). 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. This prompted a study in which PTLD patients failing a reduction of immunosuppression were given four weekly cycles of rituximab followed by CHOP as sequential treatment (ST). In 70 patients, the ORR was 60% following rituximab, which increased to 90% with ST with CHOP. With a median follow-up of 5 years, the median TTP and median PFS were 77 months and 48 months, respectively. The 5-year PFS was 66% and 5-year OS was 57% (Trappe et al., 2011). The TRM with CHOP was 11%. Of note, three patients had PCP and thus, prophylaxis became mandatory. This trial was amended in 2007 to introduce risk stratification so that patients in a CR after rituximab monotherapy were considered low risk and received four further courses at 3-week intervals, whereas patients not in a CR received R-CHOP for four cycles with G-CSF support (risk-stratified sequential treatment [RSST]). An interim analysis of 40 patients treated with this approach and 64 patients treated with the ST approach described previously was reported at the 2009 American Society of Hematology meeting. With a median follow-up of 34 months in the ST group and 9 months in the RSST group, the ORR after rituximab monotherapy was 54% (32% CR), increasing after the CHOP or R-CHOP chemotherapy (Trappe et al., 2009). With RSST the ORR was 90% (CR 73%), and 90%

were without disease progression at 1 year; there was one death due to infection (2.5%). In the ST group, 86% and 75% were without disease progression at 1 year and 2 years, respectively, and the early TRM was 9%. Comparing the ST and RSST arms, there was one event in each suggesting that consolidation with rituximab in RSST for CR patients seems comparable to sequential consolidation with CHOP. Conversely, patients who are not in a CR appear to benefit from sequential (R)-CHOP chemotherapy. Reduced immunosuppression and single-agent rituximab are reasonable first-line treatments in the majority of patients with close surveillance and sequential therapy with R-CHOP in those who do not achieve a CR. For patients who present with very high-risk aggressive disease, R-CHOP can be considered front-line treatment with G-CSF support with strong consideration also for PCP prophylaxis.

Mantle cell lymphoma

In many ways, MCL falls between the indolent and aggressive lymphomas, unfortunately combining the poorer attributes of each, namely, the lack of curability with standard therapy and a relatively aggressive clinical course. With better recognition of MCL as a unique entity, and treatment strategies developed specifically for MCL, the median OS of MCL appears to be improving, now longer than 5 years (Hermann et al., 2009).

The clinical features of MCL include median age of 64, a striking male predominance, and advanced stage at presentation frequently with bone marrow and peripheral blood involvement. Extranodal involvement is present in the majority of cases, with 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 by biopsy.

Cytologically, the majority of 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, including small cell, which is 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 that of a B-cell lymphoma, and in addition, they are typically CD5+, FMC7+, and CD43+ but CD10- (Table 21-2). Some of the salient features that distinguish MCL from SLL or CLL are the expression of cyclin D1 and FMC7 and the lack of CD23 expression (Table 21-2). 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 technique. This reciprocal translocation juxtaposes the immunoglobulin heavy-chain locus and the cyclin D1 (*BCL-1*) 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%–29% (intermediate), and >30% (worst). With regards to clinical factors, the IPI does not provide adequate prognostic usefulness when applied to MCL, leading to the generation of an MCL-specific index (Hoster et al., 2008). The MCL international prognostic index (MIPI) identified four clinical features: age, PS, LDH, and WBC as independently associated with OS (Table 21-7). The MIPI score can separate patients into three risk groups and is quite valuable for characterizing patients on a clinical trial. It is not always useful in clinical practice, as older age and poor PS may classify a patient as “high risk,” but such patients are not necessarily conducive for therapy intensification.

Management of newly diagnosed MCL

There is no “standard” therapy or approach to MCL. It is a relatively uncommon lymphoma subtype (6% of new cases), making comparative trials difficult to conduct. A small number of cases have a behavior similar to the indolent lymphomas and a period of observation is reasonable. Most patient’s have symptomatic aggressive disease and require treatment. There are a variety of phase II studies in the literature and only recently were the first randomized clinical trials reported by the European MCL Network. This group has adopted a strategy of separating patients by age and designing trials using intensive treatment strategies for younger patients (defined as age 65 or less) and nonintensive strategies for older patients (defined as age 60 or more). The 5-year overlap is intentional to allow patients between the ages of 60 and 65 to be candidates for either approach, depending on comorbidities. This strategy is a useful one for clinical practice.

For the younger patient with MCL, several intensive strategies appear to produce comparable results. The first intensive strategy to gain widespread application was the R-HyperCVAD with alternating R-MTX/cytarabine, pioneered by investigators at the M.D. Anderson Cancer Center (Romaguera et al., 2005). This single-institution study enrolled 99 patients with a median age of 61 years. The

approach produced response rates >95% and long-term follow-up revealed a 5-year PFS of ~50% (Romaguera et al., 2010). Older patients on this trial have not fared as well, with a median PFS of ~3 years and substantially more toxicity. Another intensive approach that generated highly promising results comes from the Nordic Lymphoma Study Group. They tested an intensive induction immunochemotherapy with alternating cycles of “maxi” R-CHOP and rituximab plus cytarabine followed by *in vivo* purge (with rituximab) and ASCT in a phase II trial. The study was limited to patients <age 65 years and the median age was 56 years. The ORR was 96%, and the 6-year EFS and OS were 56% and 70%, respectively. Long-term follow-up demonstrated a continuing pattern of relapse, suggesting cures are not likely, even with this approach. The European MCL Network has presented results of large phase III randomized clinical trial in MCL patients <65 years (Hermine et al., 2010). This trial compared the efficacy of six courses of R-CHOP followed by myeloablative radiochemotherapy and ASCT versus alternating courses of R-CHOP/R-DHAP followed by a high-dose cytarabine containing myeloablative regimen and ASCT. The study was designed to test the contribution of cytarabine in the management of younger MCL patients (median age 56 years). The 3-year PFS was significantly better in the cytarabine-containing arm (75% vs. 60%), and with longer follow-up, an OS advantage recently was reported at the American Society of Hematology 2012 meeting (median OS not reached vs. 82 months, $P = .045$).

Until recently, there were no trials focusing on the ~50% of MCL patients who are not candidates for an intensive therapy approach. The European MCL Network conducted a trial for patients >60 years, who were assigned randomly to induction with either R-CHOP or the R-FC (rituximab, fludarabine, cyclophosphamide) regimen (Kluin-Neilmans et al., 2012). Responding patients underwent a second randomization to maintenance therapy with rituximab (MR) or interferon- α (IFN α), each 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, and that R-CHOP plus MR-treated patients experienced improved 4-year OS compared with R-CHOP plus IFN-treated patients (87 vs. 63%, $P = 0.005$),

respectively. This trial indicates that R-CHOP followed by MR is a reasonable front-line standard for older MCL patients.

BR is a viable alternative to R-CHOP as induction therapy for the older MCL patient. A large randomized trial that compared BR with R-CHOP in patients with newly diagnosed indolent and MCL lymphoma was reported updated at the 2012 ASCO meetings (Rummel et al., 2012). For the entire study population, the BR was better tolerated than the R-CHOP, with less alopecia, neutropenia, and infections. Regarding the MCL patients, there were 45 randomized to BR and 48 randomized to R-CHOP. The median age was 70 years. In a planned subgroup analysis, BR was superior to R-CHOP for median PFS (35 vs. 22 months, $P = 0.006$).

Management of relapsed MCL

Younger patients relapsing after intensive therapies are candidates for alloSCT. The literature varies widely in the efficacy of this approach, but it 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 allogeneic SCT in relapsed MCL. For older patients, the BR regimen is highly active in relapsed MCL, with ORR of 75%-92% reported in two small studies. The proteasome inhibitor bortezomib is FDA approved for relapsed MCL and has moderate 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 (Hess et al., 2009). The immunomodulatory agent lenalidomide has shown promise in relapsed MCL. In an international trial involving 57 MCL patients, the ORR was 42% and the median PFS was 5.7 months. Lenalidomide may be even more active when combined with rituximab. A phase I/II trial in relapsed MCL reported an ORR of 57% and median PFS of 11.1 months. Radioimmunotherapy has been evaluated in relapsed MCL, with an ORR of 31% and median EFS of 6 months (Wang et al., 2009). Perhaps most promising are new classes of agents that interfere with signaling through the B-cell receptor pathway. GS-1101 (formerly known as CAL-101) is an oral phosphoinositide 3 kinase (PI3 kinase) inhibitor that demonstrated an ORR of 48% in 21 MCL patients treated on a phase I study (Kahl et al., 2010). In a preliminary analysis of an ongoing phase II trial, ibrutinib, an oral Bruton's tyrosine kinase (BTK) inhibitor, demonstrated an ORR of 69% in 51 patients with relapsed MCL (Wang et al., 2011).

Peripheral T-cell lymphomas

PTCLs represent 10%-15% of all NHLs in Western populations and are a heterogeneous group of mature T-cell neoplasms arising from post-thymic 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 is now well established but is a relatively recent advance. A recent large retrospective study, the International Peripheral 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 (Vose et al., 2008).

Given disease rarity, there are no randomized controlled trials establishing that an alternate regimen is superior to CHOP, and thus CHOP remains the standard therapy of PTCLs. There is a range of disease in this category (Table 21-3), and a minority have a more favorable prognosis or a more indolent course.

Indolent PTCLs

Mycosis fungoïdes and Sézary syndrome

In contrast to nodal NHL which are mostly B-cell derived, ~75% of primary cutaneous lymphomas have a T-cell phenotype and two-thirds are either Mycosis fungoïdes (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 similar to indolent B-cell lymphomas, it is incurable. 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 are 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 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 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 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%-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 (reviewed Prince et al., 2009). 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 phototherapy 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 poor and transient. Combination chemotherapy regimens are not particularly effective and provide only transient responses (Prince et al., 2009). Single-agent treatment is 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 good single-agent activity. Alternatively, IFN α , bexarotene, vorinostat, and denileukin diftitox all have efficacy in advanced-stage MF and SS. 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%). Denileukin diftitox is a recombinant fusion protein that combines interleukin 2 (IL-2) with the cytotoxic A chain of diphtheria toxin with an ORR of 49%. It is approved by the FDA for patients with relapsed CTCL whose tumors express the IL-2 receptor subunit (CD25). Histone deacetylase inhibitors prevent histone acetylation, thus altering the gene expression of cell-cycle and apoptotic regulatory proteins. Suberoylanilide hydroxamic acid (SAHA; vorinostat) and romidepsin (depsipeptide) both are approved for the treatment of CTCLs. Vorinostat is orally available and has an ORR of ~30% and a median duration of response (DoR) of ~6 months. A phase II trial with Romidepsin (depsipeptide) demonstrated an ORR 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 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. A low-dose subcutaneous alemtuzumab was investigated in 14 patients in relapsed or refractory SS and 9 out of 10 patients achieved a response using this schedule, including three CRs. Importantly, infectious complications were not observed.

Allogeneic transplant has been explored in select cases of MF and SS. The European Group for Blood and Marrow Transplantation recently reported a multi-institutional retrospective study evaluating alloSCT (myeloablative and RIC) in 60 patients with MF ($n = 36$) or SS ($n = 24$). Almost half had refractory disease at the time of alloSCT and 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%, $P = 0.006$).

Large-cell transformation in MF is defined as large cells in >25% of the infiltrate or if these cells form microscopic nodules. The incidence ranges from 8% to 39% and typically is associated with a poor prognosis, but long-term survivors can be seen. A recent study evaluated 100 cases of transformed MF and the median survival was 2 years with a 5-year OS and disease-specific survival (DSS) of 33% and 38%, respectively. 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%-33% in patient's with three or four factors. The optimal management is unclear, but for young patients, systemic chemotherapy should be used and consideration should be made for autologous or allogeneic transplantation particularly with high-risk disease. Consolidative radiation may be considered in local transformation.

Primary cutaneous ALCL

Primary cutaneous ALCL (C-ALCL) is part of a spectrum of diseases in 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% (Bekkenk et al., 2000). It is notable that patients with localized C-ALCL with one draining lymph node involved have a similarly good prognosis. For localized C-ALCL, surgery with or without radiation is the preferred therapy as the impact of chemotherapy is unknown. 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 conservatively in minimally symptomatic patients with radiotherapy for a few lesions or low-dose methotrexate, similar to the management of lymphomatoid papulosis. If a more aggressive behavior is observed, multiagent chemotherapy is reasonable.

Given that C-ALCL is CD30+, anti-CD30 antibodies have been tested. SGN-30 demonstrates an ORR of 82%. The antibody drug conjugate (ADC) brentuximab vedotin (SGN-35) described in the following section, is being evaluated in C-ALCL, and given its efficacy in systemic ALCL, it should be highly effective in C-ALCL.

Primary cutaneous CD4⁺ small/medium T-cell lymphoma

This is a new provisional entity in the updated WHO classification and is characterized by the presence of localized plaques or nodular lesions that most commonly occur on the face, neck, or upper trunk. Epidermotropism is uncommon and, if present, is focal. The malignant cells are CD3+, CD4+, and CD8⁺ and may be accompanied by a loss of pan-T-cell markers. The prognosis is excellent, with a 5-year OS of 80%. Localized lesions typically are treated with surgery with or without radiotherapy.

T-cell large granular lymphocytic leukemia

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 and $20 \times 10^9/L$. The T-cells are CD3+, CD8+, and CD57/CD16 and are expressed in most cases, but CD56 is negative. It arises more commonly in rheumatoid arthritis or other autoimmune disorders. Most cases have an indolent clinical course, with a median survival time of ~13 years, but rare cases with an aggressive course also have been described. Of note, T-LGL should be distinguished from NK-cell leukemia, which does have a fulminant aggressive course (see the following section Aggressive NK-Cell Leukemia). In T-LGL, moderate splenomegaly is the most common clinical finding, and lymphadenopathy is rare. Severe neutropenia

with or without anemia is common, but thrombocytopenia is rare. 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, cyclosporine A, cyclophosphamide, chlorambucil, or corticosteroids can be effective. Responses can take up to 4 months and longer therapy often is needed to maintain the response. Purine analogs have been used in the relapsed setting. Splenectomy may be useful in 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 (ORR 50%).

Aggressive PTCLs

Adult T-cell leukemia/lymphoma

Adult T-cell lymphoma/leukemia (ATL) is caused by infection with human T-cell leukemia virus type 1 (HTLV-1) and occurs in endemic areas of infection (eg, Caribbean basin and southwestern Japan). The cumulative incidence of adult T-cell lymphoma/Leukemia 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, 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 either the acute (13%) or lymphoma-type (87%) ATL. Opportunistic infections are common and strongyloides serology is recommended before starting therapy.

Survival time in the acute and lymphomatous variants are ~6 and ~10 months, respectively. The median survival time 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. The IPI was prognostic in the ITLP, although only 18.5% were in the good prognosis category.

Asymptomatic patients with the smoldering or chronic types can be monitored closely. For young, fit patients with the acute and lymphoma subtypes, the intensive regimen 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 III trial comparing the dose-intensive regimen VCAP/AMP/VECP versus CHOP-14 alone and showed more favorable CR rate (40% vs. 25%, $P = .02$) and 3-year OS (24% vs. 13%) that was significant after adjusting for prognostic factors but only for the one-sided P -value ($P = .028$) (Tsukasaki et al., 2007). 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 patients should be referred for consideration for transplant.

A number of phase II studies have evaluated the anti-retroviral zidovudine (AZT) and IFN with response rates up to 92% and median OS of 11 months in untreated patients. For the leukemia subtype, these results are superior to what is achieved with combination chemotherapy. For the chronic and smoldering type, a recent 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 CC4 and a recently reported phase II study demonstrated an ORR of 50%, including eight CRs, in 27 patients treated patients. The median PFS and OS were 5.2 months and 13.7 months, respectively (Ishida et al., 2012). The most common side effects were lymphopenia (96%), neutropenia (52%) and thrombocytopenia (52%), infusion reaction (89%), and skin rashes (63%). This agent is also being explored in other CCR4+ CTCL and PTCLs.

PTCL-not otherwise specified, systemic anaplastic large cell lymphoma, ad angioimmunoblastic T-cell lymphoma

PTCL-not otherwise specified (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, representing 66% of all PTCL cases.

PTCL-NOS

PTCL-NOS is the most common subgroup of PTCLs, accounting for up to 30% of cases worldwide. This 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%-30% in most series. Recognizing that the division of PTCLs into leukemic, nodal, and extranodal is somewhat artificial, these categories have been eliminated in the updated WHO classification (Table 21-3). 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 downregulated, and ~30% are CD30+. Gene expression profiling has been explored in heterogeneous PTCL-NOS to determine whether there are reproducible, molecular subsets and to better define prognostic markers within PTCL-NOS. In comparison with B-cell lymphomas, however, large-scale studies are lacking.

Treatment approaches in PTCL have paralleled those DLBCL; as a result, CHOP is considered the standard therapy, despite consistent evidence that it is rarely curative. Furthermore, because of disease rarity, most studies have combined all subtypes that could obscure benefits in select subtypes. Limited analyses suggest that the use of anthracyclines, a key component of CHOP, may not affect outcome in PTCL-NOS (Vose et al., 2008). Although the limitations of CHOP are fully recognized, there is no clear evidence that any other approach is superior. Furthermore, given disease rarity, randomized trials are sparse. The GOELAMS (Groupe Ouest Est des Leucémies et des Autres Maladies du Sang or Western and Eastern Group of Leukemias and other Blood Diseases) group compared alternating VIP (etoposide, ifosfamide, cisplatin)/ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) to standard CHOP for eight cycles and found no difference in EFS or OS. The 5-year EFS in the CHOP-treated group in the setting of a clinical trial population was 35% (Simon et al., 2010).

The DSHNHL group retrospectively analyzed the outcome of PTCL patients ($n = 331$) that had been enrolled in phase II or phase III aggressive lymphoma studies and evaluated the impact of etoposide. In patients <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 only a trend to improved 3-year EFS (61% vs. 48%; $P = .057$), with no OS difference observed; however, patient numbers were small. Given that this is not a randomized comparison, the true benefit of the addition of etoposide remains unknown.

Alemtuzumab selectively targets CD52, which is present on normal T-cells but expression across PTCLs is more variable. In the initial studies of alemtuzumab in relapsed or refractory PTCLs, the ORR was 36%, but the TRM was also 36% because of profound immunosuppression and

opportunistic infections that can occur. Several studies have evaluated alemtuzumab with CHOP-21 or CHOP-14 in the management of PTCL with variable results. Toxicity has been problematic, including opportunistic infections. There have been reports of EBV-positive lymphoproliferative disorders secondary to the immunosuppression. The best results were observed a phase II study from the *Gruppo Italiano Terapie Innovative nei Linfomi (GITIL)*, which evaluated alemtuzumab with CHOP on a extended 4-week schedule. The toxicity was improved, but opportunistic infections still occurred, including pulmonary aspergillosis. The 2-year FFS was 48% in contrast to a study from the HOVON (Stichting Hemato-Oncologie voor Volwassenen Nederland) group, which demonstrated a 2-year EFS of only 27% (Kluin-Nelemans et al., 2011). Phase III studies are ongoing to determine whether the addition of alemtuzumab improves outcome in PTCL.

With the disappointing results with CHOP, some groups are evaluating new chemotherapy combinations in PTCL. Gemcitabine has shown reasonable single-agent activity in previously treated patients and is being explored with other agents. A number of novel agents are under investigation in PTCLs.

Angioimmunoblastic T-cell lymphoma

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.

Patients are typically in their sixth or seventh decade and have advanced-stage disease, often with B-symptoms and hepatosplenomegaly. It was originally believed to be a form of immune dysregulation, with polyclonal gammopathy and other hematologic abnormalities (Coombs-positive hemolytic anemia) reflecting B-cell hyperactivity. Opportunistic infections can occur because of the underlying immunodeficiency.

Survival is similar to PTCL-NOS (5 year ~30%); however, a small proportion may have a more indolent course. CHOP typically is used for the primary therapy and although the response rate is high, relapse is common and infectious complications are problematic. GELA evaluated AITL patients enrolled on different therapeutic protocols and found no improvement of survival with any therapy, including HDC/ASCT. With the presence of EBV-infected B-immunoblasts and the evidence of B-cell hyperstimulation, GELA also recently evaluated R-CHOP in AITL in a phase II study. Of

25 evaluable patients, the ORR was 80% (CR 44%) but with a median follow-up of 2 years, the 2-year PFS was only 42%, which was similar to a prior study using CHOP alone. With poor outcomes using conventional therapy, immunomodulatory agents also have been explored, including cyclosporine, lenolidomide, thalidomide, and interferon. A retrospective study evaluating cyclosporine in relapsed or refractory AITL demonstrated an ORR 67% and a median DoR of 13 months.

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, primary systemic ALCL is separated from C-ALCL, and more recently, ALK-positive ALCL has been defined as a distinct entity (Table 21-3). 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%-85% of all systemic ALCL, with higher frequencies seen in the pediatric and young adult age-groups. In contrast, although ALK-negative ALCL lacks any defining features, there is accumulating evidence that it should be separated from other PTCLs; as a result, it is considered a provisional entity in the updated WHO classification.

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 pre-rituximab treatment era. The improved outcome in part is related to the young age 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 patient's <40 years old, 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 <40 years of age, there was no impact of ALK status on PFS or OS (Sibon et al., 2012).

Given the favorable outcome with anthracycline-based chemotherapy, CHOP is considered to be the standard therapy of ALK-positive ALCL. Patients with multiple IPI factors have a poor outcome, however, and could be considered for clinical trials. Given the strong and uniform expression of CD30, the ADC brentuximab vedotin has been tested in the relapsed or refractory setting and has significant efficacy, prompting evaluation in the frontline setting.

ALK-negative ALCL

Patients with ALK-negative ALCL tend to be older at presentation; the clinical presentation is similar to ALK-positive cases, but sites of extranodal disease may vary. Pathologically, it is not reproducibly distinguished from ALK-positive ALCL other than lacking the ALK protein. ALK-negative ALCL has been difficult to define, in part due to a lack of uniformly applied diagnostic criteria across studies. Recently, a recurrent balanced translocation t(6;7)(p25.3;q32.3) has been identified in ALK-negative ALCL, but the significance is unknown. Previously, it was argued that ALK-negative ALCL had a similar outcome to PTCL-NOS and they should be grouped together. In recent years, there is accumulating evidence 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%, $P = .032$). These data confirm that ALK-negative ALCL should be considered distinct from both ALK-positive ALCL and PTCL-NOS. Although the survival is more favorable than PTCL-NOS, it is still poor, particularly with multiple IPI factors. Novel therapies, including brentuximab vedotin, are being explored.

ALK-negative ALCL associated with breast implants

Concerns have been growing about a possible association of breast implants with ALK-negative ALCL. ALCL associated with implants typically involves the capsule without invasion of the breast tissue, or it presents as an unexplained seroma or mass, which usually is ALK negative. The neoplastic cells float in the effusion fluid or become embedded tissue; importantly, however, breast parenchyma usually is not identified and the ALCL cells are at distance from the breast tissue. It appears to be associated with textured breast implants and expanders. Recently, investigators at the University of Southern California have collected 90 cases to date worldwide and put forth recommendations (Brody et al., 2012). A total capsulectomy should be performed, and because bilateral cases have been reported, removal of the uninvolved breast implant should be

considered. The growing body of literature supports that ALK-negative ALCL in this setting appears to have a more indolent clinical course, and most patients can be observed following removal of the implant and capsule. Recent reports suggest similar survival compared with those who received chemotherapy or radiation, but rare aggressive cases have been reported. Cases that have identified a distinct breast mass may be better classified as a typical systemic ALK-negative ALCL and may be treated accordingly (Aladily et al., 2012).

Extranodal NK-/T-cell lymphoma, nasal-type

Extranodal NK-/T-cell lymphomas, nasal-type, display great variation in racial and geographic distribution, with the majority of cases occurring in the Far East. Patients are typically males, 40–50 years old. The tumor cells show angioinvasion and necrosis is prominent. The designation NK/T is used to reflect the fact that although most 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 often is measured, providing prognostic information and disease monitoring. The majority of cases remain localized with <20% presenting with advanced-stage disease. Despite the predominant nasal location, spread to the CSF is very uncommon. Most occur in the nasal region, but identical tumors also can occur at extranasal sites, such as the 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 was ~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%; Group 4 3 or 4 RF, 5-year OS 7%. Risk factors identified in other studies have also included local tumor invasion (tone or skin), high Ki-67, or EBV DNA titer $>6.1 \times 10^7$ copies/mL.

Accumulating evidence supports that radiotherapy is critical 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 front-line setting. Recently, the use of platinum

as a radiosensitizer has been explored 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% (Kim et al., 2009). Similarly, concurrent radiotherapy (50 Gy) and DeVic chemotherapy (dexamethasone, etoposide, ifosfamide, carboplatin) was evaluated in a phase I/II trial in localized nasal NK-/T-cell lymphoma with good results (CR 77%, 2-year PFS 67%) (Yamaguchi et al., 2009). In the absence of a randomized trial, the most recent NCCN guidelines suggest either high-dose radiotherapy alone (>50 Gy) for stage 1 patients without risk factors (as described) 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 extremely 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 II 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 (Jaccard et al., 2011). A phase II study evaluating the SMILE regimen (steroid, methotrexate, ifosfamide, L-asparaginase, etoposide) in 38 patients with either newly diagnosed stage IV or relapsed or refractory NK-/T-cell lymphoma demonstrated an ORR after two cycles of 79% (CR 45%) and 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%. Additional studies incorporating L-asparaginase in the frontline treatment of both localized and advanced-stage NK-/T-cell lymphoma are ongoing.

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 more 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 and extranodal NK-/T-cell lymphoma but require further study.

Rare Aggressive PTCL subtypes

Subcutaneous panniculitis-like T-cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma (SCPTCL) is an extremely uncommon PTCL subtype that preferentially infiltrates the subcutaneous tissue. Recently, it has been determined that tumors with $\gamma\delta$ phenotype have a far inferior prognosis to those with $\alpha\beta$ phenotype (5-year OS, 11% for $\gamma\delta$ vs. 82% for $\alpha\beta$) (Willemze et al., 2008). In the WHO classification, SCPTCL is confined only to the $\alpha\beta$, 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 “Primary cutaneous PTCL, rare aggressive subtypes”) because of similar aggressive behavior. The optimal therapy for $\alpha\beta$ SCPTCL is unknown with durable responses observed with both CHOP and immunosuppressive agents.

Hepatosplenitic T-cell lymphoma

Hepatosplenitic T-cell lymphoma is a rare PTCL subtype occurring usually in young men (median age 34 years) presenting with hepatosplenomegaly and bone marrow involvement. Up to 20% of hepatosplenitic T-cell lymphomas occur in the setting of immunosuppression, most commonly following solid organ 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 will show 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 with rare long-term survivors. The optimal therapy is unknown; however, CHOP does not appear to cure this disease. Long-term survivors have been reported with high-dose chemotherapy and ASCT or alloSCT and referral at diagnosis is suggested.

Enteropathy-associated T-cell lymphoma

Enteropathy-associated T-cell lymphoma (EATL) is a rare, aggressive intestinal tumor with a male predominance that often occurs in the setting of celiac disease. It most commonly involves the jejunum or ileum. Patients often present with abdominal pain, and intestinal perforation can occur.

The prognosis is extremely poor due to chemotherapy resistance and difficult treatment delivery related to abdominal complications that can arise in the setting of malabsorption. In some cases, there is a childhood history of celiac disease, but more commonly, the disease occurs in adulthood. Alternatively, there is a prodrome of refractory disease, or a concomitant diagnosis of celiac disease is found at the time the lymphoma is discovered. In the updated WHO classification, a sporadic, monomorphic variant, type II EATL, has been defined that occurs in 10%-20% of cases and has a broader geographic distribution that includes Asia. An association with celiac disease has not been definitively proven in this subtype; thus, this may represent a distinct disease entity. In the common subtype, the neoplastic cells are CD3⁺, CD7⁺, CD4⁻, CD8^{-/+}, CD56⁻ and contain cytotoxic proteins. The monomorphic form is CD3⁺, CD4⁻, CD8⁺, and CD56⁺.

The ITLP recently reported on 62 patients with EATL, which represented 5.4% of all lymphomas worldwide, most commonly in Europe. Type I and type II EATL 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 “Transplant in PTCL”).

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.

Transplant in PTCL

Multiple retrospective studies have been published evaluating the impact of upfront transplantation in PTCL, as has been comprehensively reviewed (Yared et al., 2012). Trial interpretation and comparisons are difficult for a number of 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 III 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.

GELA performed a retrospective analysis of the impact of upfront autologous transplant in T-cell lymphomas. Limiting the study to patients who achieved CR, a matched-pair analysis was performed comparing dose-intensive chemotherapy alone (ACVB or NCVB (mitoxantrone substitution) versus chemotherapy plus HDC/ASCT. No difference in DFS or OS was found, but the ACVB is considered more dose-intensive than CHOP.

Several phase II prospective studies of upfront transplant have been published and represent more homogeneous populations of treated patients. The Nordic group completed the largest prospective phase II trial of upfront transplant (NLG-T-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), followed by BEAM/BEAC and ASCT in responding patients (d’Amore et al., 2012). 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-transplant was 81% to transplant and the overall transplant 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 AILT (49%), but this was not statistically significant. The 5-year OS for patients who underwent transplant was 61% compared with 28% in those who did not. These results suggest that this approach maybe appropriate in select patients but still represent level 2 evidence given the absence data from a phase III trial.

In eligible patients, HDC/ASCT represents the standard of care for relapsed or refractory PTCL. 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 PTCLs was small. There has been no

similar randomized study in PTCLs, but a number of retrospective studies report a salvage rate in this setting ranging from 18% to 60% (Yared et al., 2012). Given the overall body of evidence, ASCT frequently is offered to patients with PTCL with relapsed, chemosensitive disease.

AlloSCT, with either myeloablative or RIC, also has been reported to yield durable remission in many cases (3-year EFS 23%-64%). Evidence supporting a graft-versus-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 II trial evaluating RIC and alloSCT 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 toxicity related to graft-versus-host disease.

Novel PTCL therapies

A number of agents are being explored in PTCL, 3 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 II 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 DoR of 10.5 months (O'Connor et al., 2011). 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 are ongoing combining pralatrexate with other agents in the up-front and relapsed settings.

As described previously romidepsin (depsipeptide or FK228) is a HDAs that has been evaluated in CTCLs and PTCLs. A phase IIB registration study recently 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. Side effects were as previously described in the CTCL studies. A phase Ib study is ongoing combining CHOP with romidepsin for the primary treatment of PTCL.

CD30 is expressed uniformly in ALCL but 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, and thus to enhance tumor activity an antibody-drug conjugate (ADC), brentuximab vedotin (SGN-35), was developed. The ADC conjugates the CD30 monoclonal antibody to the microtubulin

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 II study recently was reported in relapsed or refractory systemic ALCL that demonstrated an ORR 86% (CR 57%), median DoR 12.6 months, and a median PFS 13.3 months, which also prompted FDA approval for this disease in 2011. The main side effect of brentuximab vedotin is peripheral neuropathy. Studies are ongoing evaluating brentuximab vedotin in the upfront setting with CHP, omitting the vincristine because of overlapping toxicity.

A number of others agents currently are being evaluated in clinical trials in PTCL, including bendamustine, lenolidomide, bortezomib, and denileukin difitox.

NHLs in children

NHLs account for 8% of cancers in children. There are striking variations of incidence by sex (male-to-female ratio of 2:3:1) and race (white-to-black ratio of 2:1, rare in Asian populations). There is an increased incidence in patients with congenital (ataxia-telangiectasia, Wiskott-Aldrich syndrome, and others) or acquired (posttransplantation) immunodeficiency states. Prior EBV infection has been implicated in the pathogenesis of endemic (African) BL but is less common in sporadic American and European cases.

The histologic subtypes of childhood NHL are significantly different from those in adults. BL (mature B-cell) account for one-third of cases, lymphoblastic lymphomas (primarily T-cell) account for 30%, and large-cell lymphomas of multiple lineages account for 25%-30%. FLs are rare in children, but chromosomal translocations and molecular features are similar to those in adults with the same lymphoma subtype.

Childhood NHLs are staged using the Murphy staging system. Therapy depends on the stage. Ninety percent of patients with stage I or II Burkitt or large-cell lymphoma can be cured with 9 weeks of CHOP-like chemotherapy. Using the same regimen, only 70% of patients with stage I or II lymphoblastic lymphoma are cured. Adding a 1- to 2-year continuation phase of treatment with methotrexate, mercaptopurine, vincristine, and prednisone may decrease the relapse rate, although this has not been definitively established. CNS prophylaxis may be limited to patients with lower stage NHL who have head and neck primary sites.

Stage III and IV (initial involvement of the CNS or bone marrow) lymphomas require more intensive chemotherapy. Burkitt and large B-cell lymphomas can be cured in 80%-85% of cases with 3-8 months of chemotherapy that includes cyclophosphamide, doxorubicin, dexamethasone, vincristine, high-dose methotrexate, and high-dose cytarabine. A

similar protocol that also includes ifosfamide and etoposide has cured almost 80% of children with advanced-stage ALCLs. Lymphoblastic lymphomas are cured in a similar fraction of cases with 2-3 years of treatment with protocols similar to those for higher risk acute lymphocytic leukemia in children (see Chapter 17). CNS chemoprophylaxis with intrathecal medications is an important component of treatment of all subtypes in children with advanced-stage NHLs. Some protocols still use low-dose (12-18 Gy) cranial irradiation as part of CNS prophylaxis for patients with advanced lymphoblastic lymphomas.

Key points

- DLBCL is the most common subtype of aggressive NHL in Western populations; T- and NK-cell subtypes are more common in Asian populations.
- R-CHOP-21 remains the standard treatment for DLBCL and results in cure in ~50%-60% patients with DLBCL.
- The IPI and cell of origin phenotype remain prognostic in the rituximab treatment era. Studies are ongoing whether patients classified as high risk by the IPI or ABC phenotype should be treated with an alternate therapy other than R-CHOP.
- Relapsed aggressive lymphoma patients who have chemotherapy-sensitive disease to a second-line regimen (at least a PR) should undergo treatment with HDC/ASCT.
- BL should be treated with dose-intensive regimens as R-CHOP is inadequate. The treatment should 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 exception is ALK-positive ALCL, which has a high cure rate with CHOP chemotherapy unless multiple IPI factors are present at diagnosis.
- Most children with NHL have aggressive subtypes; however, high cure rates are seen with multidrug chemotherapy regimens.

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Chronic lymphocytic leukemia

Vicki A. Morrison and Grzegorz S. Nowakowski

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CHAPTER
22

Chronic lymphocytic leukemia

Vicki A. Morrison and Grzegorz S. Nowakowski

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Introduction

Incidence, epidemiology, and demographics

Chronic lymphocytic leukemia (CLL) is one the most prevalent lymphoid malignancies. The disease is identical to small lymphocytic lymphoma (SLL), an indolent B-cell non-Hodgkin lymphoma. The incidence of CLL in the United States are 6.75 and 3.65 cases per 100,000 population per year in males and females respectively, resulting in a male to female ratio of 1.7:1. The incidence of the disease shows significant geographic differences. The disease is considerably less common in the approximately Far East than in Western countries. Genetic and environmental factors may play a role in geographic differences, since the incidence in the Asian population in the United States appears to be lower as well. CLL incidence is higher among Caucasian than African Americans and Asians and Pacific Islanders. There are no clearly identifiable environmental factors known to predispose to CLL.

The incidence of CLL increases with age, with a median age at diagnosis of 70 years. The disease rarely is diagnosed in people <30 years of age. Although the disease in younger and elderly patients is phenotypically indistinguishable, age may have a significant impact on selection of therapy (see the section on therapy).

Familial CLL

Approximately 5%–10% of CLL patients have a family history of CLL and other lymphoid malignancies. First-degree family

members of patients with CLL are at increased risk of CLL, monoclonal B-cell lymphocytosis, and other lymphoid malignancies. It appears that familial CLL tends to present at a younger age, with a higher proportion of affected females than in the general population. Familial CLL is phenotypically and biologically indistinguishable from sporadic CLL. Currently, there are no known genetic factors predisposing to CLL. Screening of family members outside of clinical studies is not recommended.

Key points

- CLL is more common in Western countries.
- The incidence of CLL increases with age.
- CLL can be a familial disease.

Clinical presentation

Diagnosis

CLL is identical to SLL, and these two entities represent opposite ends of the spectrum of the disease. Although patients with CLL present with lymphocytosis, sometimes extreme, patients with SLL present with predominantly nodal or extranodal disease without or with minimal blood involvement (<5,000 monoclonal B-cells/ μ L [$5 \times 10^9/L$], see also the section International Workshop on CLL Criteria for Diagnosis). Diagnosis of CLL can be established based on a review of peripheral blood and peripheral blood flow cytometry. No single genetic abnormality or molecular marker would be specific for a diagnosis of CLL, although recurrent genetic alterations can be seen and have prognostic value (see also the section Differential Diagnosis: Cytogenetic, Immunophenotypic, and Molecular Aspects). Peripheral blood smears show

Conflict-of-interest disclosure: Dr. Morrison: speakers bureau: Amgen; membership on board of directors or advisory committee: Celgene, Merck. Dr. Nowakowski declares no competing financial interest.

Off-label drug use: Dr. Nowakowski will discuss lenalidomide, ibrutinib, everolimus, flavopiridol and GS1101.

a population of morphologically mature-appearing small lymphocytes with often prominent fragile or “smudge” cells (Figure 22-1). Peripheral blood flow cytometry demonstrates monoclonal population of B-cells with a characteristic immunophenotype: low levels of surface immunoglobulin, either κ - or λ -light chains; expression of CD19, CD20, and CD23; and aberrant expression of CD5. CD10 is usually negative (see also the section Differential Diagnosis: Cytogenetic, Immunophenotypic, and Molecular Aspects).

Bone marrow biopsy or lymph node biopsy is not required to establish the diagnosis, as CLL diagnosis can be established solely based on peripheral blood flow cytometry and blood smear. If a lymph node biopsy is performed in CLL patients, it shows findings consistent with SLL—that is, predominantly composed of small lymphocytes with condensed chromatin and round nuclei. Larger lymphoid cells (prolymphocytes) are present and clustered in pseudofollicles often referred to as proliferation centers, which is a characteristic feature of SLL/CLL.

International Workshop on CLL criteria for diagnosis

Because there is a continuum of the disease between SLL and CLL, for classification purposes, the International Workshop on CLL developed guidelines on the diagnosis and treatment of CLL and established criteria for CLL diagnosis. Two of the following are required:

- Absolute B-cell count in the peripheral blood $\geq 5,000/\mu\text{L}$ ($5 \times 10^9/\text{L}$) for at least 3 months, with a preponderant population of morphologically mature-appearing small

lymphocytes. Because the definition is based on absolute B-cell count, not absolute lymphocyte count, flow cytometry assessment of B-cell number is required.

- Demonstration of clonality of the circulating B-cell by flow cytometry with a characteristic phenotype: low level of surface immunoglobulin, either κ - or λ -light chain expression, CD5+, CD19+, CD20+(dim), CD79b+(dim).

Patients with a monoclonal B-cell population $< 5,000/\mu\text{L}$ ($5 \times 10^9/\text{L}$) and without evidence of lymphadenopathy or extranodal involvement are characterized as patients with monoclonal B-cell lymphocytosis (MBL, see also the section Monoclonal B-cell Lymphocytosis). Patients with lymphadenopathy or extranodal involvement histologically proven to be consistent with SLL, with an absolute peripheral B-cell count that is $< 5,000/\mu\text{L}$ ($5 \times 10^9/\text{L}$) are given the diagnosis of SLL rather than CLL (Table 22-1).

The cutoff of 5,000 B-cells in the current definition is arbitrary, and there is controversy regarding the ideal cutoff and its clinical significance. In clinical practice, many patients present with a high number of lymphocytes at diagnosis, which facilitates a diagnosis of CLL.

Table 22-1 Classification of CLL, SLL, and MBL.

Diagnosis	B-cell count	Lymphadenopathy/extranodal involvement
CLL	$\geq 5,000$	+/-
SLL	$< 5,000$	+
MBL	$< 5,000$	-

CLL = chronic lymphocytic leukemia; SLL = small lymphocytic lymphoma; MBL = monoclonal B-cell lymphocytosis.

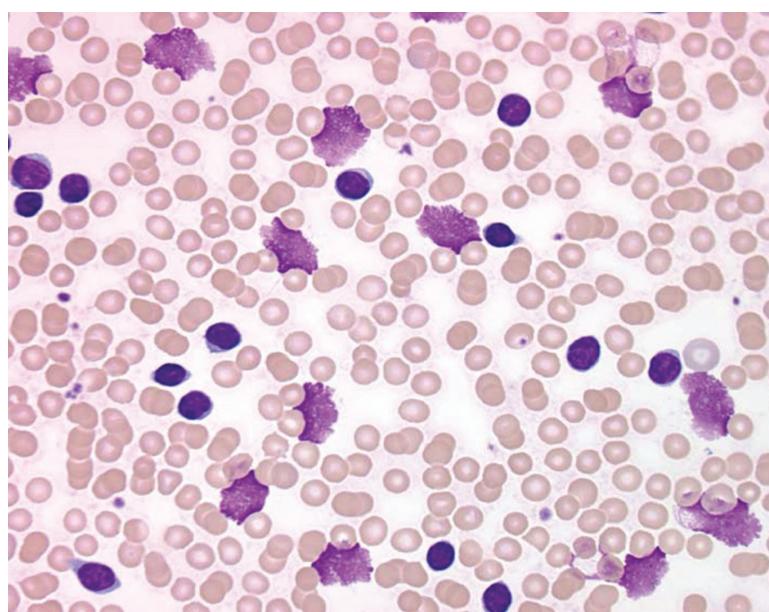


Figure 22-1 Peripheral blood smear of patient with CLL. Increased number of mature small to medium lymphocytes can be seen with presence of fragile or smudge cells. Although smudge cells are not pathognomonic for CLL, presence of ruptured cells is often a prominent feature, and the high number of smudge cells has been associated with improved prognosis.

Differential diagnosis: cytogenetic, immunophenotypic, and molecular aspects

With the use of interphase fluorescent in situ hybridization (FISH) techniques preferentially performed on peripheral blood samples, >80% of CLL patients will be found to have a chromosomal abnormality. The most common aberrations are deletion 13q14 (55% of cases), deletion 11q22-23 (18%), trisomy 12 (16%), and deletion 17p13 (7%). Other defects less commonly found (<10% of cases) include deletion 6q, total or partial trisomy 3, and 14q32 translocation. The incidence of these chromosomal defects increases with advancing disease stage. In addition, new defects may be acquired in clonal evolution of the disease process. More than a decade ago, a prognostic model for disease progression and survival based on cytogenetic parameters was developed. Patients with 13q deletion as the sole cytogenetic abnormality were found to have the most favorable prognosis, followed by those with no defects detected by FISH, trisomy 12, and 11q deletion; those patients with 17p deletion had the worst prognosis. Associated genes involved with these defects include TP53 (deletion 17p13), ataxia telangiectasia mutated (ATM) gene (deletion 11q22-23), and the micro-RNA genes miR15a and miR16-1 (two micro-RNAs) (deletion 13q14).

Characterization of the CLL immunophenotype by peripheral blood flow cytometry is used to establish the diagnosis and to differentiate the disease process from other B-cell lymphoproliferative disorders (Table 22-2). CLL cells generally coexpress CD19, a pan B-cell marker, CD20 (with dim expression), CD23, and CD5 (a T-cell marker), and they typically have dim light chain and CD79b expression. CD22 is variably expressed. The low affinity anti-CD20 antibody FMC7 is usually negative, as are CD10, CD103, and cyclin D1. Surface immunoglobulin expression (generally immunoglobulin M [IgM], or IgM plus immunoglobulin D [IgD], either κ or λ) is dim. In contrast, mantle cell lymphoma cells usually have higher levels of expression of CD20 (FMC7 positive) and light chains, and do not express CD23. Surface immunoglobulin is expressed, and CD23 expression is variable, in marginal zone lymphoma, in contrast to dim-surface immunoglobulin and consistent with CD23 expression in CLL cells.

Table 22-2 Chronic B-cell lymphoproliferative disorders: prototypic immunophenotype.

	sIg	CD20	CD5	CD23	CD10	CD103
Chronic lymphocytic leukemia	Dim	Dim	+	+	-	-
Lymphoplasmacytic lymphoma	Mod	+	-/+	+/-	-	-
Mantle cell lymphoma	Mod	+	+	-(partial)	-	-
Marginal zone: Nodal/MALT lymphoma	+	+	-	-/+	-	-
Splenic marginal zone lymphoma	+	+	-/+	-/+	-	-/+
Follicular lymphoma	+	+	-	-/+	+/-	-
Hairy cell leukemia	+	+	-	-	-	+

Data regarding the molecular disease aspects are being reported increasingly. Patients with a somatically hypermuted immunoglobulin variable heavy chain (IGHV) gene (55%-65% of cases) usually have a more indolent disease and a more favorable prognosis than those patients with unmutated IGHV genes. Deletions in 17p are associated with the loss of one allele of p53, conferring a poor prognosis. Bcl-2 protein, which suppresses apoptosis and is associated with hypomethylation of the promoter region of the Bcl-2 gene, is found in 85% of cases. CD38 expression as well as ZAP-70 expression may be detected by flow cytometric techniques and may be utilized in combination with IGHV gene mutational status to predict disease prognosis. Molecules such as ang-2, which are involved in angiogenesis, also are being examined with regard to prognostic impact.

Monoclonal B-cell lymphocytosis

MBL is defined as a monoclonal B-cell population that does not exceed 5,000/µL ($5 \times 10^9/L$) in a patient who does not have lymphadenopathy, organomegaly, cytopenias, or disease-related symptoms. Per definition, MBL requires flow cytometry to document monoclonal B-cell population. In clinical practice, MBL frequently is diagnosed during: (i) evaluation of asymptomatic patients with mild, incidentally noted lymphocytosis; or (ii) flow cytometric analysis of blood of patients with normal lymphocyte count done for other reasons. The majority of patients with MBL will have a CLL immunophenotype (CD19, CD5, and CD23 positive and dim expression of CD20, CD79b, and immunoglobulin light chain), but this is not a requirement for diagnosis. Using multicolor flow cytometry, about 3% of healthy people have a clonal population of B-cells in their blood (MBL). The incidence of MBL increases with age and is found in ~5% of patients >60 years of age with normal blood counts and 14% of patients >60 years of age with lymphocytosis. Several recent studies have found that ~1%-2% of people with MBL and lymphocytosis will progress to CLL per year. Conversely, a monoclonal B-cell population appears to be present years before the diagnosis in virtually all patients with CLL. Interestingly, for individuals with MBL who have

a CLL immunophenotype, the monoclonal B-cells frequently exhibit cytogenetic abnormalities and other biomarkers typically seen in CLL (see the section Novel Prognostic Factors). The role of these biomarkers in predicting MBL progression to CLL remains largely unknown. Importantly, most people with MBL will never develop CLL or other lymphoid malignancies. Although the MBL clone may disappear overtime, little is known about the rate of spontaneous resolution of MBL. There are no known interventions that can prevent development of CLL. Although most experts recommend yearly follow up, there are no standard evidence-based guidelines in regard to follow-up of individuals with MBL.

Key points

- There is no single genetic abnormality diagnostic for CLL; however, cytogenetic aberrations are common in CLL patients and predictive of prognosis.
- CLL cells are CD19, CD20, CD5, and CD23 positive, with dim-surface immunoglobulin and CD20 expression. This immunophenotypic pattern distinguishes CLL from other B-cell disorders.
- A diagnosis of CLL can be established from the peripheral blood. A bone marrow biopsy is not required for diagnosis.
- MBL is defined as a monoclonal B-cell population $<5,000/\mu\text{L}$ ($5 \times 10^9/\text{L}$) in the absence of other finding suggestive of a lymphoproliferative disorder.
- MBL with lymphocytosis is associated with 1%-2% annual risk of progression to CLL.

Clinical and laboratory features

CLL often is diagnosed incidentally, when a complete blood count (CBC) done for other purposes reveals an absolute lymphocytosis, and immunophenotyping demonstrates a monoclonal B-cell population $>5 \times 10^9/\text{L}$ with a CLL immunophenotype. Patients generally present with symptoms referable to lymphadenopathy, splenomegaly, or anemia, fatigue, and recurrent infections. Only a minority of patients will present with classical B-symptoms (fever, night sweats, weight loss). On physical examination, localized or diffuse lymphadenopathy may be present, as well as hepatomegaly or splenomegaly.

The predominant laboratory feature with this disease is the peripheral blood mature lymphocytosis. Smudge or basket cells are common on the peripheral blood smear. Fewer than 10% of patients will have mild anemia (hemoglobin $<11\text{ g/dL}$) or thrombocytopenia (platelet count $<100 \times 10^9/\text{L}$) at diagnosis. A relative neutropenia is also common. Approximately 25% of patients will have or develop a positive direct Coombs test (DAT) over the course of their disease. Likewise,

over time, an autoimmune hemolytic anemia or thrombocytopenia may occur in 10%-20% of patients. Hypogammaglobulinemia is a hallmark of this disorder, with increasing prevalence and severity with advanced disease stage and longer disease duration. A serum monoclonal paraprotein (usually IgM) is present in <5% of cases. The bone marrow is generally normocellular or hypercellular, with involvement in either a nodular, interstitial, or diffuse pattern.

Key points

- At presentation, CLL patients are often asymptomatic; some may present with lymphadenopathy, hepatosplenomegaly, or classical B-symptoms (fever, night sweats, weight loss).
- A predominance of mature lymphocytes and smudge cells are seen on the peripheral blood smear.
- Hypogammaglobulinemia is a hallmark of this disease.

Prognostic factors

Traditional prognostic factors

The clinical course of patients with CLL is variable, ranging from an indolent process to one with a more accelerated course. Before the advent of cytogenetic and molecular markers, a series of traditional prognostic factors were recognized. The Rai and Binet staging systems correlate with median survival, which was initially reported as >13 years with Rai stage 0 disease, 8 years for stage I, 6 years for stage II, 2-6 years for stage III, and 1.5-4 years for stage IV disease. In a more recent update from the Mayo Clinic, median overall survival (OS) in patients with Rai stage III or IV disease was 5-6 years. It has also been suggested that in patients with early stage disease, which constitutes ~ 70% of the CLL population at diagnosis, an elevated β -2 microglobulin level ($>3.5\text{ mg/L}$), CD38 expression, degree of lymphocytosis, and serum thymidine kinase (sTK) levels are predictive of the clinical course. A lymphocyte doubling time (LDT) of <12 months, compared with >12 months, also is associated with a shorter median survival. A diffuse pattern of bone marrow involvement also correlates with a poorer prognosis than a nodular or interstitial pattern of involvement. Other poor prognostic factors include male gender, initial lymphocytosis of $>50 \times 10^9/\text{L}$, elevated serum lactate dehydrogenase (LDH), African American ethnicity, number of nodal groups involved, and advanced age, in some but not all, series.

Molecular prognostic factors

Approximately 70% of patients with CLL are diagnosed at early Rai stage disease. Consequently, although Rai staging

system remains clinically useful, particularly in assessing need for initiation of therapy, it does not provide risk stratification for the majority of patients diagnosed at early stage. This has led to an effort to identify novel biomarkers able to risk stratify patients with early clinical stage disease. In addition to OS, the frequently used endpoint in biomarker studies is time from diagnosis to initial therapy (TTT). A number of prognostic factors have been identified, and there is an overlap in their prognostic value, as many were developed as surrogates of earlier biomarkers to overcome some of the technical difficulties in assessing these. For practical purposes, prognostic markers commonly in use can be divided in four major categories: mutational status of IGVH, cytogenetic studies, flow cytometry-based markers, and mutations in TP53 gene. A number of prognostic models combining these markers have been or are in development. Outside of clinical trials, however, the presence of adverse biomarkers is not an indication for initiating therapy in patients with early Rai clinical stage disease. As of now, biomarkers do not affect selection of therapy, with possible exception of deletion of 17p deletion, which is associated with resistance to purine analogues.

IGVH mutation status

IGVH undergoes somatic hypermutation in patients with CLL that can be detected by direct sequencing. Patients with ≤98% homology of IGVH gene with germline DNA are characterized as “mutated,” whereas those with >98% homology are considered “unmutated.” Approximately 50%-55% of patients have unmutated IGVH. In contrast to cytogenetic markers, the mutational status of IGVH does not change during the course of the disease. The median survival of patient with unmutated versus mutated IGVH is 5-10 years versus 10-20 years. The biological reasons for these differences in outcomes are not understood, but data do suggest a role for antigen stimulation through unmutated IGVH. Differences in the origin of the CLL cells also have been postulated. Although IGVH mutation status is not readily available in many clinical laboratories, it has been reported in many clinical studies, and it remains an important biomarker in ongoing studies.

CD38 and ZAP70

Expression of CD38 as evaluated by flow cytometry is associated with shorter time to initial therapy and shorter OS. The correlation between unmutated IGVH and CD38 expression is about 70%. In contrast to IGVH mutation status, CD38 expression may change in some patients during the course of the disease.

ZAP70 is an intracellular tyrosine kinase that is expressed aberrantly in CLL. Expression of ZAP70 is associated with

inferior outcome with median survival of 8-9 years versus 24 years for patients who do not express this protein. The correlation between unmutated IGVH and ZAP70 expression is about 70%-80%, and the prognostic value of ZAP70 expression appears to be independent of IGVH mutation status.

Fluorescent in situ hybridization

A low proliferative rate of CLL in culture precludes classical cytogenetic studies in most patients. In contrast, FISH can be performed on interphase cells and can identify abnormalities in 80% of patients. The studies of FISH detected abnormalities allow development of hierarchical prognostic model (Figure 22-2). Deletion of 13q14 is the most common and is seen in 45%-55% of patients. A deletion of 13q14 as a sole abnormality is associated with favorable outcome.

In contrast, deletion of 17p13 is associated with poor outcome with median time from diagnosis to treatment of 9 months and median OS of 32 months. Indeed, presence of 17p13 appears to be associated with resistance and shortened progression-free survival (PFS) after purine analogue-based therapies (see the section Therapy). 11q22 deletion is associated with shorter PFS and OS. Patients with 11q22 deletion tend to present at a younger age with predominance of extensive adenopathy. Some studies suggested that patients with 11q22 deletion may benefit from the addition of alkylator to the therapy. The prognostic value of trisomy 12 is well less defined. Patients with trisomy 12 appear to have similar outcome to patients with normal karyotype. The cytogenetic abnormalities may change in the course of the disease in significant proportion of patients (clonal evolution). Particularly, acquisition of 17p deletion in patients with accelerated course of the disease may be seen and repeating FISH testing in patients with rapid acceleration of disease is recommended. Similarly, repeated 17p testing should be considered in previously observed patients who are to initiate therapy because of progressive disease.

CLL cells can be stimulated in vitro to enter the cell cycle through a number of stimuli, allowing for classical karyotype analysis. Complex karyotypes detected by utilizing this approach have been reported to be associated with worse outcomes. This method, however, is not used routinely in a clinical setting.

TP53 mutations

TP53 is a tumor-suppressor gene located on the short arm of chromosome 17 (17p13). It can be inactivated by deletion (17p13) and by somatic mutations within the gene. TP53-inactivating mutations have been identified in 5%-10% of CLL patients and are associated with shortened OS and

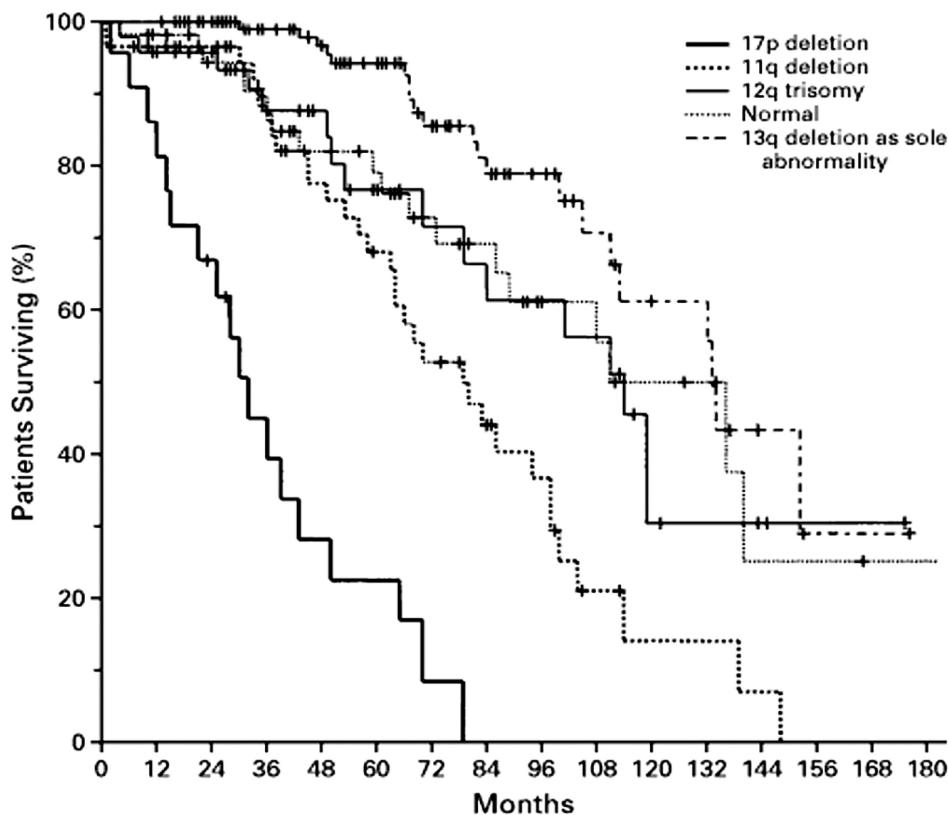


Figure 22-2 Probability of survival from the date of diagnosis among the patients in the five genetic categories. The median survival times for the groups with 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as the sole abnormality were 32, 79, 114, 111, and 133 months, respectively. Twenty-five patients with various other chromosomal abnormalities are not included in the analysis. Dohner H, et al. *N Engl J Med*. 2000;343:1910-1916.

inferior response to therapy in a manner that is similar to TP53 inactivation by 17p deletion.

Prognostic models and ultra-high-risk patients

The development of novel prognostic biomarkers led to an effort to combine those in a number of prognostic models. Although many of these proposed prognostic models will require verification in prospective studies, there appear to be groups of patients with particularly high-risk disease. In this regard, the presence of 17p deletion or TP53-inactivating mutations regardless of IGVH mutation status and status of other biomarkers identifies group of patients at high risk of progression and death from the disease. In addition to these abnormalities, in the relapsed disease setting, patients within the ultra-high-risk group are defined as those having disease progression <24 months after purine analogue-containing chemoimmunotherapy (CIT) and those refractory to purine analogue therapy. There is no evidence that early therapy of ultra-high-risk patients (eg, not meeting standard criteria for initiation of therapy) is beneficial (see the section Therapy). These patients should be strongly considered for participation in clinical trials, including trials designed for high-risk early stage disease. In the case of younger patients with PFS of <24 months following purine-based therapy or purine analogue refractoriness, allogeneic stem cell transplantation (SCT) should be considered (see the section Stem Cell Transplantation).

Key points

- Prognosis of CLL patients at diagnosis may be assessed by traditional clinical and laboratory-based parameters, as well as by newer clinical and molecular diagnostic techniques.
- Models incorporating traditional and novel biomarkers are now available to aid in counseling patients on clinical outcome.
- The bone marrow may be normo- or hypercellular, with CLL involvement in a nodular, interstitial, or diffuse pattern.
- Some patients with early stage disease (Rai stage 0, Binet stage A) may progress more rapidly than others.
- Expression of CD38 or ZAP70 and unmutated IGVH are associated with shortened PFS and OS.
- Deletion of 17p and TP53 inactivating mutations are associated with particularly aggressive disease and refractoriness to therapy.

Therapy

Initial therapy

Approaches to initial CLL therapy have evolved over the past several decades (Table 22-3). Alkylators were the backbone of therapy for many years. In the 1990s, purine analogues were introduced, and in the following decade, they became the backbone of CIT approaches, with the addition of such agents as rituximab and cyclophosphamide. Over the past decade, the utility of alemtuzumab was established, followed by the

Table 22-3 CLL clinical staging systems.

Binet classification			Rai classification			Median overall survival, y	
Stage	Definition	Patients (%)	Risk group	Stage	Definition	Patients (%)	
A	<3 lymphoid areas	60	Low	0	Lymphocytosis only	30	>10
B	>3 lymphoid areas	30	Intermediate	I	Lymphadenopathy	25	5–7
				II	Hepato- or splenomegaly ± lymphadenopathy	25	
C	Hemoglobin <10 g/dL or platelets <100 × 10 ³ /dL	10	High	III	Hemoglobin <11 g/dL	10	1–3
				VI	Platelets <100 × 10 ³ /dL	10	

Two systems have been used widely to classify stage of progression of CLL: Rai and Binet staging.

They define early (Rai 0, Binet A), intermediate (Rai I/II, Binet B), and advanced (Rai III/IV, Binet C) stages of CLL on the basis of the extent of lymphoid areas involvement and on the presence of anemia and thrombocytopenia.

Kokhaei et al. *Ann Oncol*. 2005;16(S2):ii113-ii123.

introduction of bendamustine. Unfortunately, recommendations for initial therapy predominantly are based on published phase II trials, instead of phase III comparison trials.

Alkylator-based therapy

Alkylator-based therapy, generally chlorambucil and less commonly cyclophosphamide, was the mainstay of therapy for many years, given as single agents or with corticosteroids, despite no demonstration of a survival advantage. Chlorambucil may be administered in various schedules, including daily (0.1 mg/kg/d) and pulse intermittent dosing (0.4–1.0 mg/kg, every 3–4 weeks). Reduction in lymphadenopathy, organomegaly, and symptoms occurs, with overall response rates (ORR) ranging from 38% to 75%, but with complete remission (CR) rates ranging from only 3% to 5%. Toxicities include myelosuppression as well as the potential for therapy-related dysplasia with long-term usage. This therapy is still utilized for many elderly or poor performance status patients because of its tolerability. In addition, preliminary results of phase II chlorambucil plus rituximab trials in older patients have demonstrated the efficacy and tolerability of this combination.

Fludarabine

Although fludarabine, 2-chlorodeoxyadenosine, and pentostatin have been utilized for CLL therapy, fludarabine has the widest usage. All cause defects in cell-mediated immunity occur early in treatment and persist for up to a year after discontinuation of therapy. In phase II studies, ORR range from 40% to 65%, with CR rates of 15%–30%. The addition of prednisone to fludarabine resulted in no improved response rate, but opportunistic infections did occur. Several large prospective randomized trials compared fludarabine to alkylator-based regimens for the initial

CLL therapy. Fludarabine resulted in higher ORR and CR rates, as well as prolonged remission durations of PFS, and OS. The most common toxicities are myelosuppression, fever, and infections. An oral preparation of fludarabine with similar efficacy to the intravenous form is now available.

Rituximab

The anti-CD20 antibody rituximab has gained widespread usage in the therapy of lymphoproliferative disorders, including CLL. As a single agent, ORR approach 50%, but with <5% CRs. This may be related to dim CD20 expression on the malignant lymphocyte. Single-agent rituximab has been studied in a dose-escalated manner, with slightly higher response rates seen. Weekly rituximab has been combined with high-dose methylprednisolone (1 g/m², days 1–3) for a nonmyelosuppressive, although immunosuppressive, therapy option. This regimen has demonstrated efficacy in patients with 17p deletions.

Combination purine analogue-based chemo- and chemoimmunotherapy

Fludarabine subsequently was incorporated into combination chemotherapy or CIT regimens to enhance both short- and long-term outcome. Fludarabine plus cyclophosphamide (FC) had been compared with single-agent fludarabine in three large randomized phase III trials. In all, FC resulted in an improved ORR and CR rate, and prolongation in PFS, but not in OS, and it also resulted in more myelosuppression. As only a small number of elderly patients were enrolled on these trials, tolerability data in these patients are limited.

Fludarabine plus rituximab (FR) has been studied in concurrent and sequential schedules. In a randomized

phase II study, concurrent administration resulted in a higher CR rate than sequential therapy (47% vs. 33%), but with more neutropenia but no increased infection rate. When these results were compared with those of single-agent fludarabine in the U.S. intergroup trial, FR demonstrated greater efficacy, with improved ORR (84% vs. 63%), CR rate (38% vs. 20%), 2-year PFS (67% vs. 45%), and 2-year OS (93% vs. 81%). Now with long-term follow-up, median PFS and OS are 42 and 85 months, respectively, and 27% PFS at five years. IGVH mutational status was prognostic for both PFS and OS; cytogenetic abnormalities were prognostic only for OS.

The fludarabine, cyclophosphamide, rituximab (FCR) combination regimen has been examined in multiple large trials. In the initial phase II trial with a median follow-up of 6 years, ORR and CR rates were 95% and 72%, respectively. Median time to progression was 80 months, with 6-year failure-free survival and OS of 51% and 77%, respectively. In addition, molecular remissions were demonstrated in >40% of patients achieving a CR. In this single-institution setting, toxicities, including infectious complications, were manageable. Only 13% of patients were >70 years of age, however. In a subsequent trial of the German CLL Study Group, FCR was compared with FC (CLL8). In this trial, the addition of rituximab resulted in a survival advantage. Three years after initiation of therapy, 65% of FCR, compared with 45% of FC patients, were free of progression. Although grade 3/4 neutropenia was more common with FCR than FC (34% vs. 21%), the rate of severe infections was comparably low in both groups. The FCR-lite regimen, consisting of lower dose cyclophosphamide and fludarabine and higher dose rituximab has been studied, with ORR and CR rates of 100% and 79%, respectively, with median response duration of 22%, median PFS of 5.8 years, and only 13% grade 3/4 neutropenia. The FCR regimen also has been studied with each agent given sequentially, with favorable results. The FCR regimen has a significant impact on minimal residual disease (MRD), with low MRD levels during and after therapy being associated with longer PFS and OS. The IGVH mutational status does not appear to affect the CR rate; however, CR duration is shorter in patients with unmutated IGVH status. The prospective U.S. intergroup trial comparing the FCR and FR regimens has just completed accrual. This trial also examines the issue of maintenance lenalidomide therapy.

Mitoxantrone has been added to the FCR regimen for patients ≤70 years of age in several phase II trials, with ORR of 93%-96% and CR rates of 82-83%, with some patients achieving MRD negativity. Treatment toxicities have been acceptable and comparable to that with FCR therapy. The addition of epirubicin to FR therapy resulted in similar ORR and CR rates, but myelosuppression was considerable.

Other purine analogues

The two other purine analogues, pentostatin (deoxycoformycin) and cladribine (2-chlorodeoxyadenosine [2CDA]), have been studied in CLL patients. Therapy with the combination of pentostatin with cyclophosphamide and rituximab (PCR) resulted in an ORR of 91% with 41% CR, and no evidence of MRD in some patients attaining a CR. Although efficacy was less in patients with deletion 17p, it demonstrated activity in those with deletion 11q22. Efficacy was comparable in patients less than and older than 70 years, as well as in those patients with modest decreases in renal function. Median remission duration was 36 months in CR patients. The regimen was well tolerated with no excess morbidity. In a follow-up study of higher dose pentostatin plus rituximab (PR), the ORR (76% vs. 91%), CR rate (27% vs. 41%), and median treatment-free survival (16 vs. 30 months) were inferior to results with PCR.

Cladribine (2CDA) monotherapy has had efficacy similar to alkylator-based regimens, but with more cytopenia and immune suppression. When subcutaneous cladribine was given with rituximab, ORR was 88% with 54% CR, with a median time to failure (TTF) of 38 months, and was well tolerated. In a phase III study, cladribine plus cyclophosphamide was compared with FC, with comparable results (ORR 88% vs. 82%, CR rate 47% vs. 46%, respectively). Grade 3/4 toxicities, PFS, and OS were comparable with both regimens.

Alemtuzumab

Alemtuzumab, an anti-CD52 monoclonal antibody, is highly effective as in eradicating peripheral blood and marrow disease and less effective against bulky nodal disease. It may be administered by either a subcutaneous or intravenous route, both being associated with a significant risk of infectious complications, especially cytomegalovirus (CMV) reactivation. Approval for initial therapy with this agent was based on results of a phase III study in which patients were randomized to initial therapy with alemtuzumab or chlorambucil, with ORR 83% versus 56%, and CR rates 24% versus 2%, respectively. CMV reactivation occurred in 11% of alemtuzumab subjects.

Alemtuzumab also has been a component of combination regimens. In a series of TP53-deleted CLL patients, therapy with alemtuzumab plus high-dose methylprednisolone resulted in an ORR of 88%, 65% CR rate, median PFS of 18 months, and median OS of 39 months. Although effective in this high-risk population, toxicity was considerable with grade 3/4 toxicities of myelosuppression (67%), infection (51%), and 5% treatment-related mortality. With the addition of alemtuzumab to FCR in patients <70

years of age, ORR was 92% with a 70% CR rate, including a 57% CR rate in patients with 17p deletion. Grade 3/4 neutropenia and thrombocytopenia were 33% and 13%, respectively.

This agent also has been utilized for consolidation therapy in multiple past trials, as MRD negativity may be achieved with its use. When it was used after initial therapy with fludarabine alone or with cyclophosphamide, one trial was stopped because of severe infections. In those patients who received this agent, however, PFS was prolonged as compared with those who received no consolidation. In another trial, in which 5 weeks of alemtuzumab consolidation followed six cycles of FR induction therapy, although ORR, CR, and MRD-negativity rates were high (90%, 57%, 42%, respectively), five infectious deaths occurred in patients in CR after FR.

Bendamustine

Bendamustine is a bifunctional agent with an alkylating group and a purine-like benzimidazole ring. When compared with chlorambucil in a phase III study, ORR and CR rates were higher with bendamustine (68% vs. 31%, 31% vs. 2%, respectively), and median PFS also was prolonged (22 vs. 8 months). This agent was well tolerated, with the most common toxicity being hematologic, and with hypersensitivity reactions infrequently seen. In a phase II study of bendamustine plus rituximab (BR), ORR was 88% (23% CR), which is inferior to purine analogue-based CIT regimens. At 18-month follow-up, 76% of patients were still in remission. The ORR approached 90% in patients with 11q deletions or trisomy 12. ORR dropped to 43% in those with 17p deletions, however. In an ongoing phase III study, BR is being compared to FCR for frontline therapy.

Ofatumumab

Ofatumumab is a fully human CD20 antibody that targets a membrane epitope that is different from the binding site of rituximab. In a phase II study, this agent was combined with fludarabine and cyclophosphamide (O-FC), with O being administered at either 500 or 1,000 mg. The ORR and CR rate were 77% and 32%, respectively, for the 500 mg dose cohort, and 73% and 50% for the 1,000 mg cohort. Grade 3/4 toxicities included neutropenia (48%), thrombocytopenia (15%), anemia (13%), and infection (8%). In a preliminary report of a phase II trial of pentostatin, cyclophosphamide, and ofatumumab (PCO), ORR was 94% (CR 45%), similar to results with PCR, and the regimen was well tolerated. Ongoing phase II trials with this agent include its single-agent use as induction and maintenance therapy, as well as in combination with chlorambucil and FC.

Lenalidomide

Lenalidomide is an immunomodulatory agent with activity in CLL that may be related to alteration of cytokine levels and T- and natural killer cell function. In a single-agent study, a 2.5 mg daily dose for 21 of 28 days was utilized, with monthly dose escalations up to a daily dose of 10 mg. The ORR was 56% with no CR, with an 88% incidence of tumor flare and a 72% incidence of grade 3/4 neutropenia. Although lymphocyte counts fell rapidly, rebound was common in the week off of therapy. In another study of patients >65 years of age, a 5 mg daily dose was utilized for 8 weeks, with subsequent dose titration to 25 mg daily as tolerated. The ORR and CR rate were 65% and 10%, respectively, with an estimated 2-year PFS of 60%. Grade 3/4 neutropenia and infections occurred in 34% and 13% of patients, respectively. In a small phase I trial of lenalidomide plus FR, the regimen was found not to be tolerable because of idiosyncratic drug reactions, tumor flare, and myelosuppression. The occurrence of tumor flare, which can be managed with anti-inflammatory agents, appears to correlate with disease response. In preliminary study, this agent appears to have activity in patients with poor-risk cytogenetic features.

Proposed initial treatment algorithms

Many of the frontline regimens previously discussed have not been compared in prospective randomized studies, thus making definitive treatment algorithms problematic. The following are proposed initial therapy recommendations of the National Comprehensive Cancer Network, in order of preference:

- Age <70 years, or older patients with no significant comorbidities:
 - FCR
 - FR
 - PCR
 - BR
- Age >70 years, or younger patients with comorbidities:
 - Chlorambucil +/- rituximab
 - BR
 - Cyclophosphamide, prednisone, +/- rituximab
 - Alemtuzumab
 - Rituximab
 - Fludarabine +/- rituximab
 - Pentostatin, rituximab +/- cyclophosphamide
 - Cladribine
- Presence of 17p deletion:
 - FCR
 - FR

- High-dose methylprednisolone + rituximab
- Alemtuzumab +/- rituximab
- Presence of 11q deletion:
- Age <70 years, or older patients with no significant comorbidities:
 - FCR
 - BR
 - PCR
- Age ≥70 years, or younger patients with comorbidities:
 - Chlorambucil +/- rituximab
 - BR
 - Cyclophosphamide, prednisone, +/- rituximab
 - Reduced-dose FCR
 - Alemtuzumab
 - Rituximab
 - Pentostatin, rituximab +/- cyclophosphamide

Key points

- The treatment approach to CLL has evolved, from initial therapy with alkylator-based regimens, to single-agent purine analogue therapy, followed by CIT combination regimens.
- Alemtuzumab has been used as initial therapy, or as consolidation therapy, to attain a state of MRD. Significant infectious complications have been reported, however, in some trials with alemtuzumab consolidation.
- The purine analogues and alemtuzumab have an impact on cell-mediated immunity, resulting in unique infectious complications with implications for prophylactic antimicrobial therapy.
- The most recently approved agents for CLL therapy are bendamustine and ofatumumab.
- Considerations for age, comorbidities, and poor-risk cytogenetics are needed in choosing an initial CLL therapeutic regimen.

Relapsed/refractory CLL

There is no standard treatment of relapsed/refractory CLL. The treatment choice is based on previous treatment history, patient's factors like age and fitness and risk assessment as identified by biomarkers, particularly 17p deletion or TP53 mutations. In patients with significant benefit from initial purine-based therapy defined as PFS of 24 months or longer, repeating purine-based therapy often results in durable remissions. Although patients treated previously with alkylator-based regimens and achieving durable remission could be considered for repeated therapy with the same agent, frequently, purine-based second-line therapy is used effectively. There are limited data in regards to efficacy of repeating therapy with other agents, including bendamustine and alemtuzumab; however, retreatment in the setting of previous durable response to a given agent is reasonable.

Patients with CLL relapse <12-24 months after initial purine analogues therapy, patients with primary refractory disease to purine analogues, and patients with 17p deletion or TP53 mutations are particularly difficult to treat and should be considered for participation in clinical trials when possible or allogeneic SCT. The median survival in this group is 18-24 months. Alemtuzumab alone or in combination with high-dose methylprednisolone or rituximab has activity in patients with 17p deletion and TP53 mutations, including purine analogue refractory patients with ORR of 45%-50% and PFS of 7 months. Alemtuzumab is less effective in patients with bulky lymphadenopathy and often is associated with 11q deletion. Combination regimens using alemtuzumab with FCR (CFAR) or pentostatin and rituximab (PAR) have been developed, but these combinations can be associated with significant toxicity. Ofatumumab, a human anti-CD20 antibody has ~50% response rate in patients with fludarabine or alemtuzumab refractory disease. The responses are usually partial with median survival of 13-15 months. Bendamustine alone or in combination with rituximab (BR) is associated with ORR of ~50% in patients with relapsed/refractory CLL.

The combination of rituximab and high-dose methylprednisolone may induce significant responses in this group, especially in patients with bulky disease, but these responses are often of short duration.

Investigational agents

A number of investigational therapies have been developed, and studies on their role in the management of CLL are ongoing. Inhibitors of B-cell pathway-signaling appear promising. Bruton's tyrosine kinase (BTK) inhibitors, such as ibrutinib, can be effective in patients with refractory/relapsed CLL with no alternative treatment options. The response rate to ibrutinib in patients with heavily relapsed and refractory pretreated CLL was reported to be 50%-70%, including patients with 17p deletion. The PI3 kinase inhibitor GS1101 (CAL101) and the mTOR inhibitor everolimus as well as several other BCR pathway-signaling small molecule-targeted inhibitors currently are under investigation in clinical trials. Lenalidomide is associated with a 30%-45% response rate. Patients treated with lenalidomide frequently develop "tumor flare," which is characterized by the development of painful and swollen lymphadenopathy after the initiation of therapy. The other common side effect is myelotoxicity, requiring dose reductions or therapy interruption. Flavopiridol, a cyclin-dependent kinase inhibitor, showed significant activity and is active in patients with 17p deletion. The drug is associated with a high risk of tumor lysis and aggressive tumor lysis syndrome management is required.

Key points

- There is no standard therapy for early relapsed or purine analogue refractory CLL.
- Patients with relapsed disease who previously had a durable (>12–24 months) response to purine analogue-based CIT can be retreated with CIT provided that they have not had clonal evolution with acquisition of 17p13-.
- Patients with purine analogue refractory disease or relapse within 2 years following purine analogue therapy have poor prognosis and should be considered for clinical trials or reduced-intensity conditioning (RIC) allogeneic SCT.
- Alemtuzumab is active in patients with 17p deletion and TP53 inactivating mutations, but single-agent therapy is less effective in patients with bulky disease.
- Several novel agents show promising activity and currently are being evaluated in clinical trials

Stem cell transplantation

Hematopoietic SCT is applicable only for a minority of patients with CLL. Many patients will have an indolent disease course, and likewise, many are of advanced age or have comorbidities that make them unsuitable candidates for this procedure. SCT, however, may be considered for those patients with high-risk disease, including fludarabine resistance (nonresponse or relapse <1 year after purine analogue-based therapy), defective p53 function, or relapse <2 years after purine analogue-based therapy.

Autologous SCT should be undertaken only in the context of a clinical trial. Multiple phase II trials have demonstrated the feasibility of this procedure, with transplant-related mortality (TRM) ranging from 1% to 10%. In a recent multicenter randomized trial, previously untreated CLL patients received initial cytoreductive therapy, with those attaining a CR then randomized to further chemotherapy or autologous SCT. Although a higher response rate and longer TTP was seen in those undergoing SCT, no prolongation in OS was seen. Autologous SCT is complicated by the presence of clonal cells in the stem cell product, the possibility of preexisting myelosuppression from prior therapy that may limit stem cell mobilization, and the issue that the adverse impact of biologic markers as unmutated IGVH status is not overcome by this procedure. The development of secondary myelodysplastic syndrome or acute myeloid leukemia (AML) is of concern, with an incidence approaching 10% in several large series. A greater risk of this complication is seen with total body irradiation-containing regimens. In a series with long-term follow-up, an incidence of solid tumors up to 19% also has been reported. A pattern of continuing relapse is found in these patients, with no plateau in survival seen.

Allogeneic SCT confers the benefit of a graft-versus-leukemia (GVL) effect, thus increasing the likelihood of

long-term disease control. In a large series of myeloablative SCT, however, TRM and graft-versus-host disease (GVHD)-related mortality approached 50% and 20%, respectively. Overall, approximately one-third of patients will be cured of their disease. Although data are limited, preparative regimens with total body irradiation appear to result in better outcomes than chemotherapy-only regimens. Although autologous and allogeneic SCT procedures have not been prospectively compared, in examining series with longer follow-up intervals, although PFS was longer in the autologous recipients, there was no difference in OS.

The utilization of nonmyeloablative or RIC allogeneic SCT procedures has expanded the number of CLL patients eligible for SCT. Donor engraftment and CR rates are high with these procedures, with the benefit of a GVL effect. From a large series with 5-year follow-up, TRM rates were lower, approaching 20%-25% at 5 years, disease-free survival of 50%, and PFS of 40%. Chronic GVHD, however, may be seen in up to 75% of patients. Complication rates are higher with unrelated donors; however, CR rates are higher and relapse rates are lower, demonstrating more GVL effect in this setting. Monoclonal antibodies, such as rituximab, have been used to reduce GVHD incidence posttransplant. Likewise, alemtuzumab has been used in conditioning regimens to reduce GVHD, with the complications of delayed immune reconstitution, increased infection risk, and higher relapse rates because of reduced GVL effect.

SCT may be considered as a treatment option for patients <70 years of age. Ideally, it is undertaken in the setting of low disease burden, treatment-sensitive disease. Potential indications for SCT include: (i) patients attaining less than a CR to initial therapy, (ii) those with 17p deletions at diagnosis, (iii) the occurrence of Richter transformation, (iv) fludarabine- or alemtuzumab-refractory disease, and (v) relapse from FCR or similar therapy. At present, SCT is best considered for patients as part of a clinical trial.

Key points

- Hematopoietic SCT is a treatment option for CLL patients, albeit with limitations.
- Autologous SCT is limited by contamination of the stem cell product by the malignant clone, therapy-related myelodysplasia, a higher risk of second solid tumor malignancies, and, importantly, a continuing pattern of relapse.
- Allogeneic SCT has the advantage of enhanced GVL effect, but is limited by older patient age and high TRM.
- Nonmyeloablative allogeneic SCT procedures offer lower TRM and improved long-term survival compared with myeloablative allogeneic procedures.
- Allogeneic SCT should be considered for those patients with early (< 2 years) disease progression following purine analogue-based CIT.

Complications of CLL

Autoimmune complications

An accumulation of largely immunologically incompetent cells in CLL has a profound impact on the immune system, resulting in immune dysfunction. The dysfunction results not only in immune deficiency but also is associated with defects of immune functions resulting in immune-mediated cytopenias and impairing immune surveillance against second malignancies.

Autoimmune hemolytic anemia

Autoimmune hemolytic anemia (AIHA) develops in 4%-10% of patients with CLL. AIHA can occur at any time during the disease course with the prevalence increasing as the disease progresses. Purine analogues therapy is a risk factor for the development of AIHA. Indeed, in patients with AIHA, purine analogues should be avoided, as severe and sometimes fatal hemolysis may occur. AIHA that is associated with CLL usually is mediated by warm immunoglobulin G (IgG) antibodies, although cold agglutinins also can be seen. The diagnosis is established by documentation of anti-red cell antibodies (direct Coombs test) accompanied by other features of hemolysis, including an increase in LDH, hyperbilirubinemia, and reticulocytosis. The differential diagnosis for anemia in CLL patients includes bone marrow failure from progressive disease, AIHA, pure red cell aplasia, hypersplenism, and chemotherapy-induced anemia and bleeding. Additional difficulties result from the fact that 30%-50% of patients with CLL have a positive Coombs test in the course of their disease, which usually is not associated with significant hemolysis. Therefore, the presence of a positive Coombs test may not necessarily signify the presence of AIHA. The distinction between AIHA and anemia resulting from the suppression of hematopoiesis because of disease progression is particularly important, as the former would not necessarily require the initiation of anti-CLL therapy. In these patients, bone marrow biopsy should be performed.

The management of AIHA (and other autoimmune cytopenias) in the setting of CLL is similar to the management of idiopathic AIHA unless the CLL is progressive and also requires therapy. Steroids usually are used as first-line therapy, although rituximab has significant activity in steroid refractory or relapsed disease. A splenectomy also is used in refractory cases but frequently is ineffective. In patients with purine analogue-associated AIHA, purine analogues should be stopped and hemolysis should be treated with corticosteroids. Significant transfusion support frequently is needed because of the brisk nature of purine-induced hemolysis.

Pure red cell aplasia

Pure red cell aplasia (PRCA) is characterized by the autoimmune destruction of erythroid progenitors within the bone marrow. Profound reticulocytopenia is a characteristic feature of the disease. PRCA occurs in 0.5% of CLL patients. A differential diagnosis from bone marrow failure in the setting of progressive CLL is important. In PRCA, a bone marrow biopsy reveals a lack of erythroid progenitors with relatively intact other hematopoietic elements. In addition to the autoimmune etiology, PRCA may be associated with viral infections, mainly parvovirus B19 reactivation, and less often with CMV and Epstein-Barr virus (EBV) reactivation or infection. Ruling out of viral infections is important. In this regard, patients with CLL may not be able to mount an antibody response against parvovirus B19, and serology testing might be negative. Therefore, performing PCR for direct virus detection is preferred. The management of PRCA includes the treatment of underlying viral infection, immunosuppressive therapy with corticosteroids, or cyclosporine. Rituximab also was shown to be effective in some cases.

Immune thrombocytopenic purpura

Immune thrombocytopenic purpura (ITP) is a common feature in CLL. Similarly to AIHA, ITP needs to be distinguished from other cases of thrombocytopenia, including bone marrow failure, hypersplenism, chemotherapy and drug induced, infection associated, and disseminated intravascular coagulation (DIC). Typically, thrombocytopenia in the setting of hypersplenism is mild to moderate. Severe thrombocytopenia with large platelets on the peripheral blood smear suggests ITP; however, a bone marrow biopsy might be required to differentiate ITP from bone marrow failure. Clinically significant ITP is seen in 2%-5% of CLL patients. A combination of ITP with AIHA referred to as Evan's syndrome can be seen in up to 30% of patients with ITP. The treatment of ITP in CLL is similar to idiopathic disease with corticosteroids and intravenous immunoglobulin used in first-line therapy. Rituximab frequently is used in second-line treatment, and splenectomy can be considered in some patients. The role of thrombopoietic agents in CLL associated ITP is not well established.

Infectious complications

Infections remain a major cause of morbidity and mortality in CLL patients. The pathogenesis of infection is multifactorial, including inherent immune defects related to the primary disease process and therapy-induced immunosuppression.

The early and frequently profound onset defect in humoral immunity in patients with CLL results in hypogammaglobulinemia and an increase in risk of serious infections by encapsulated bacteria. The spectrum of infectious complications of therapy is related to the specific therapeutic agents utilized.

Alkylator-based therapy usually is associated with respiratory tract infections that usually are bacterial, caused by common Gram-positive and -negative organisms. Purine analogues and alemtuzumab cause quantitative and qualitative T-cell abnormalities. In an intergroup trial, CLL patients receiving fludarabine had more major and herpesvirus infections than those receiving chlorambucil; *Pneumocystis*, *Aspergillus*, and CMV infections were uncommon. Manifestations of herpesvirus infections may be atypical. Risk factors for infection in fludarabine-treated patients include advanced-stage disease, prior therapy, response to therapy, elevated creatinine, hemoglobin <12 g/dL, and decreased serum IgG. Treatment with alemtuzumab is complicated by frequent opportunistic infections, with CMV reactivation occurring in 10%-25% of patients. Case reports of the development of progressive multifocal leukoencephalopathy following combination CIT also are emerging. For prevention of infection, the use of immunoglobulin replacement may reduce the rate of infection, but it does not affect survival, and thus routine use is not recommended. Standard vaccinations with nonlive vaccines are recommended, although immune response may be less robust. Prophylactic antimicrobial recommendations are based on the specific therapeutic regimen. With the addition of cyclophosphamide to fludarabine-based regimens, *Pneumocystis* and antiviral prophylaxis generally is utilized. For alemtuzumab, weekly monitoring for CMV reactivation by PCR is recommended, as well as *Pneumocystis* and antiviral prophylaxis. Valganciclovir prophylaxis has demonstrated efficacy in preventing symptomatic CMV reactivation but causes considerable myelosuppression.

Richter transformation

A Richter transformation may develop over time in 3%-5% of CLL patients. The diffuse large B-cell usually (about 70%-80%) arises from the CLL cells but is a de novo second lymphoid malignancy in a minority of patients. Recent data suggest that this transformation may be more common in patients of unmutated IGVH status. Presenting manifestations include fever, rapidly increasing lymphadenopathy or hepatosplenomegaly, and markedly elevated LDH levels. Prognosis is poor, with survival generally <6 months, but long-term survival is possible with aggressive therapy in some patients.

Second malignancies

Second malignancies occur in up to 25% of CLL patients, a rate almost double that seen in the general population. Malignancies with the highest increase in rates are nonmelanoma skin cancer, melanoma, lung, head and neck, prostate, kidney, and lymphoma. The cause of death is related to these second malignancies in 7%-10% of patients. Potential explanations for this finding include chronic immunosuppression related to the CLL and its therapy, risk factors common to both disorders as environmental exposures or heritable factors, and detection bias (because of physician visits). In addition, EBV-related non-Hodgkin lymphomas have been reported following alemtuzumab therapy.

Therapy-related myelodysplasia and acute myeloid leukemia (t-MDS/AML) have been reported in these patients, often with acquired abnormalities in chromosomes 5 or 7, implicating alkylator involvement. These disorders were more common in patients receiving initial therapy with concurrent chlorambucil plus fludarabine, than with either single agent, in an intergroup trial. In another prospective trial, the cumulative incidence rates of these disorders at 7 years with initial single-agent fludarabine or fludarabine plus cyclophosphamide therapy were 4.6% and 8.2%, respectively. A 10.8% incidence of t-MDS/AML was found in a series of patients receiving fludarabine-based regimens for low-grade lymphoproliferative disorders. Prior cytotoxic therapy, as well as the inclusion of mitoxantrone in the combination regimen, increased the risk of this complication. A paratrabecular pattern of marrow involvement also has been suggested as a risk factor.

Key points

- AIHA and ITP are common complications of CLL and need to be differentiated from other cases of cytopenias.
- Purine analogue therapy is associated with development of severe AIHA, which can be fatal.
- PRCA in CLL can be associated with viral infections, including parvovirus B19.
- The spectrum of infections occurring in treated CLL patients is influenced by the specific therapy utilized.
- Purine analogues and alemtuzumab result in significant cell-mediated immune defects.
- CMV reactivation is a significant issue with alemtuzumab therapy, occurring in 10%-25% of patients.
- 3%-5% of CLL patients will develop transformation to a high-grade non-Hodgkin lymphoma (Richter transformation), manifested by rapidly increasing lymphadenopathy or hepatosplenomegaly, fever, and elevated LDH.
- Second malignancies, including lung and skin cancers, are more common in CLL patients than in the general population.
- Therapy-related myeloid disorders may complicate the course of patients receiving fludarabine-based regimens.

Other related lymphoproliferative disorders

Prolymphocytic leukemia

B-cell prolymphocytic leukemia (B-PLL) is a rare mature B-cell leukemia characterized by an accumulation of prolymphocytes, typically with the involvement of the peripheral blood, bone marrow, and spleen. By definition, prolymphocytes must compromise more than 55% of blood lymphocytes. The latter distinction is important, as many CLL patients may have some prolymphocytes seen on the peripheral blood smear. The differential diagnosis requires the exclusion of CLL, myeloid cell leukemia (MCL), and other lymphomas in the leukemic stage. B-PLL affects predominantly males with a median age of 65-70 years. Immunophenotype bright-surface immunoglobulin expression and bright CD20 expression are features that differentiate it from CLL. CD5 expression is seen in 30% of the cases, and the disease is typically CD10 negative. FISH for t(11;14) often is required to differentiate B-PLL from the leukemic stage of MCL. Patients often present with rapidly progressive lymphocytosis accompanied by the development of organomegaly and presence of systemic symptoms. Optimal treatment is not established. Alemtuzumab has been used with some success. The disease usually is fatal within several years from diagnosis.

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Plasma cell disorders

Irene M. Ghobrial and Martha Q. Lacy

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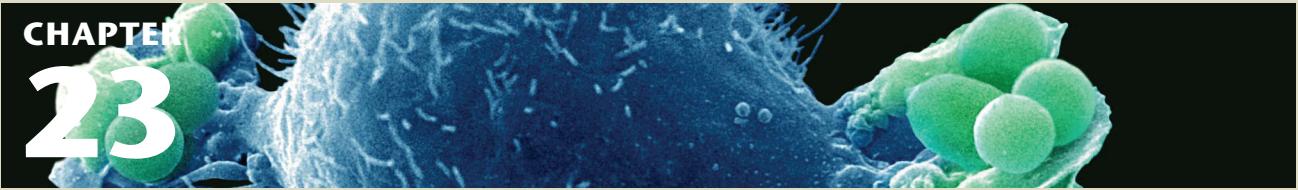
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CHAPTER
23



Plasma cell disorders

Irene M. Ghobrial and Martha Q. Lacy

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Introduction

Plasma cell dyscrasias include monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM), plasmacytoma, Waldenström macroglobulinemia (WM), amyloidosis (AL), and POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy monoclonal gammopathy, and skin changes). MGUS, smoldering MM (SMM), and symptomatic MM represent a spectrum of the same disease. MGUS is characterized by a serum monoclonal protein, <30 g/L, <10% plasma cells in the bone marrow (BM), and absence of end-organ damage. Smoldering (asymptomatic) MM is characterized by having a serum immunoglobulin (Ig) G or IgA monoclonal protein of 30 g/L or higher or 10% or more plasma cells in the BM but no evidence of end-organ damage. Symptomatic or active MM is characterized by any level of monoclonal protein and the presence of end-organ damage that consists of the CRAB (hypercalcemia, renal insufficiency, anemia, or bone lesions) criteria. Table 23-1 summarizes the diagnostic criteria of monoclonal gammopathies.

MM is a plasma cell malignancy that characteristically involves extensive infiltration of BM, with the formation of plasmacytomas, as clusters of malignant plasma cells inside or outside of the BM milieu. Consequences of this disease are numerous and involve multiple organ systems. Disruption of BM and normal plasma cell function leads to anemia,

leukopenia, hypogammaglobulinemia, and thrombocytopenia, which variously result in fatigue, increased susceptibility to infection, and, less commonly, increased tendency to bleed. Disease involvement in bone creates osteolytic lesions, produces bone pain, and may be associated with hypercalcemia. Plasmacytomas extending into soft tissue also may cause symptoms specific to the tissue involved, such as spinal cord compression. A hallmark of the disease is expression of abnormal monoclonal (M) protein, classically attributed to switch mutations in the Ig genes. M protein is secreted into the blood by malignant plasma cells in the majority of patients with MM and can contribute further to complications, including renal dysfunction, hyperviscosity syndrome, and peripheral neuropathy. Binding of M protein to plasma proteins also may lead to metabolic disturbance and contribute to clotting deficiencies. MM is characteristically diagnosed by the detection of elevated levels of M protein in the serum or urine and the presence of plasma cells in the BM. The most common presenting symptoms are bone pain and fatigue.

Plasma cell development

B-cell maturation consists of early (antigen-independent) and late (antigen-dependent) stages, ultimately terminating in the development of the plasma cell. Early development is initiated by the rearrangement of genes for the heavy and light chains of antibodies, a process referred to as V/(D)/J recombination. The earliest B-cell precursor shows rearrangement of the immunoglobulin heavy chain, which is then followed by light chain rearrangement. The κ -light chain genes rearrange first; if neither κ -locus is productively rearranged, then the λ -gene loci undergo rearrangement.

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Off-label drug use: Dr. Ghobrial: not applicable. Dr. Lacy: not applicable.

Table 23-1 Diagnostic criteria for monoclonal gammopathies.

Disorder	Disease definition
MGUS	Serum monoclonal protein level <3 g/dL, bone marrow plasma cells 10%, and absence of end-organ damage, such as lytic bone lesions, anemia, hypercalcemia, or renal failure, that can be attributed to a plasma cell proliferative disorder
SMM (also referred to as asymptomatic multiple myeloma)	Serum monoclonal protein (IgG or IgA) level ≥3 g/dL or bone marrow plasma cells ≥10%, and absence of end-organ damage, such as lytic bone lesions, anemia, hypercalcemia, or renal failure, that can be attributed to a plasma cell proliferative disorder
Multiple myeloma	Bone marrow plasma cells ≥10%, presence of serum or urinary monoclonal protein (except in patients with true nonsecretory multiple myeloma), plus evidence of lytic bone lesions, anemia, hypercalcemia, or renal failure that can be attributed to the underlying plasma cell proliferative disorder
Solitary plasmacytoma	Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells, normal skeletal survey, and MRI of spine and pelvis, and absence of end-organ damage, such as anemia, hypercalcemia, renal failure, or additional lytic bone lesions, that can be attributed to a plasma cell proliferative disorder

Ig = immunoglobulin; MGUS = monoclonal gammopathy of undetermined significance; MRI = magnetic resonance imaging;

SMM = smoldering multiple myeloma.

Adapted with permission from Rajkumar V, et al. Multiple myeloma: diagnosis and treatment. *Mayo Clin Proc*. 2005;80:1371-1382.

Once successful light chain rearrangement occurs, the cell expresses the complete immunoglobulin molecule on its surface, which identifies it as a mature B-cell. Mature B-cells typically will express either immunoglobulin M (IgM) or IgD on their surfaces, and this surface expression is critical to cell survival and maturation.

The late, or antigen-dependent, stages of B-lymphocyte development begin when a naïve B-cell recognizes an antigen with its membrane-bound antibody. These B-cells collect in germinal centers of the various lymphoid organs and begin to divide and undergo several types of genetic modification. Somatic hypermutation is a process by which cells introduce mutations into the variable region genes. These mutations result in antibodies that may have a higher or lower affinity for the antigen. Those that produce a higher affinity antibody will persist and become either plasma cells or memory B-cells, whereas those that fail to produce functional antibody at this stage will undergo apoptosis. Class switching involves changing the heavy chain that is expressed to produce other antibody classes, IgG, IgA, or IgE. Although this switch does not alter antibody affinity, the change in class of antibody will alter its effector function and thereby affect the immune response.

such as radiation and certain chemicals, as well as first-degree relatives indicating familial predisposition in some patients, although this appears rare. Several studies indicate that myeloma risk increases with cumulative exposure to ionizing radiation. Chemicals such as dioxin and other herbicides and pesticides also have been shown to increase the risk of myeloma as much as three- to fourfold. In a recent study, the prevalence of MGUS among pesticide applicators was twice that in a population-based sample of men from Minnesota, adding support to the hypothesis that specific pesticides are causatively linked to myelomagenesis.

Recent studies suggest that an asymptomatic MGUS stage consistently precedes MM. MGUS is present in 3% of persons >50 years and in 5% >70 years of age. The risk of progression to MM or a related disorder is 1% per year. For MGUS patients, a non-IgG isotype, M-protein concentration >1.5 g/dL and an abnormal free light chain (FLC) ratio (normal range 0.25-1.65) are considered adverse prognostic factors. At 20 years, the risk of progression in patients with zero, one, two, and three risk factors is 5%, 21%, 37%, and 58%, respectively. For SMM patients, an M-protein ≥3 g/dL, an FLC ratio outside the range of 0.125 to 8, and ≥10% plasma cells in the BM are considered to be adverse factors in this model. The 5-year rate of progression in patients with one, two, and three risk factors was 25%, 51%, and 76% respectively. The time to progression (TTP) with these risk factors was 10, 5.1, and 1.9 years, respectively. Recently Rajkumar et al. (2011) have proposed that SMM with >60% plasma cells progress to MM within 2 years in 95% of cases and should be treated at diagnosis even in the absence of symptoms. A study of the natural history of SMM suggests that there are two different types: evolving SMM and

Etiology and incidence

Although the etiology of myeloma and other plasma cell dyscrasias is not known, several risk factors have been identified. MM is more common among African Americans than other racial groups and is more common in men than women. Other than race and sex, they include environmental agents,

nonevolving SMM. Evolving SMM is characterized by a progressive increase in M protein and a shorter median TTP of 1.3 years. Nonevolving SMM has a more stable M protein that may then change abruptly at the time of progression, with a median TTP of 3.9 years.

The incidence of MM increases with age, with an average age at diagnosis of 65 years, and is more common in people of West African heritage, being the second most common hematologic malignancy after non-Hodgkin lymphoma. The incidence in the United States is 20,580 cases, and the estimated number of deaths is 10,580 according to the 2009 estimates.

factor receptor 3 (*FGFR3*) gene. Finally, t(14;16)(q32;q23) dysregulates the oncogene *MAF*, a basic leucine-zipper transcription factor, in 5%-10% of patients, and t(14;20) (q32;q11) affects another member of this family, *MAFB*, in 5% of cases. These rearrangements generally seem to be mutually exclusive, although in 5% of MGUS and 25% of advanced MM cases, two independent translocations may be found in the same patient. t(14;16) and t(4;14) translocations are associated with a poor prognosis, whereas t(11;14) translocations are related to longer survival time relative to all other genetic subtypes in patients with MM (Figure 23-1).

In addition to the hyperdiploid–nonhyperdiploid dichotomy and the presence of chromosomal translocations, specific gains or losses of certain chromosomal regions occur are also linked to prognosis. These include chromosome 13 monosomy, loss of the short arm of chromosome 17 (where the tumor-suppressor gene *TP53* resides) or the short arm of chromosome 1, and gains or amplifications of the long arm of chromosome 1. The oncogene *MYC* is involved in chromosomal translocations or amplifications in up to 45% of patients with advanced MM. Deletions of chromosomes 17p and 1p, as well as loss of chromosome 13, are linked to poor prognosis, although the prognostic significance of chromosome 13 remains controversial. Indeed, chromosome 13 loss by conventional cytogenetics portends poor prognosis to conventional low- and high-dose chemotherapy but not to the proteasome inhibitor bortezomib. The presence of chromosome 13 deletion by fluorescence in situ hybridization (FISH) does not significantly affect survival of MM patients. Gains or amplifications of chromosome 1q also were proposed recently as an adverse prognostic factor. In particular, in the most comprehensive expression profiling survey of MM patients published to date, Shaughnessy et al., 2007, identified 70 genes linked to early disease-related death, thereby providing the first validated classifier for prognosis prediction in uniformly treated MM patients. Strikingly, 30% of these genes were located on chromosome 1, with most of the downregulated genes located on the short arm of chromosome 1 and most of the upregulated genes on 1q.

Molecular pathogenesis

Genetic and epigenetic regulation of MM

MM can be subdivided into two groups according to the pattern of chromosomal gains and losses, hyperdiploid and nonhyperdiploid MM. Approximately 55%-60% of MM primary tumors are characterized by a hyperdiploid karyotype with a number of chromosomes ranging from 48-74 and trisomies of odd-numbered chromosomes, including 3, 5, 7, 9, 11, 15, 19, and 21. The remaining cases make up a nonhyperdiploid group, which includes tumors with a hypodiploid or near-tetraploid chromosome number (ie, fewer than 48 or more than 74 chromosomes). Patients with hyperdiploid MM tend to have a better prognosis than those with nonhyperdiploid disease. It is not fully understood the mechanisms underlying these changes but ploidy status rarely changes during disease progression. Within the hyperdiploid group, array CGH has identified a subset of patients who present with additional gains on 1q or losses of chromosome 13. These patients have a worse prognosis than do patients in the nonhyperdiploid group.

Another characteristic abnormality in MM is chromosomal translocations involving the IgH locus at 14q32.3 (heavy chain), and less frequently the IgL locus at 2p12, κ or 22q11, λ (light chain) (Table 23-2). These chromosomal translocations more often affect nonhyperdiploid patients and are linked to prognosis. Two of these translocations directly increase the expression of cyclins: t(11;14)(q13;q32), which induces cyclin D1 expression and occurs in about 15%-20% of MM patients and t(6;14)(p21;q32), which induces cyclin D3 expression and occurs in 5% of MM cases. Another translocation, t(4;14)(p16.3;q32), is present in ~15% of patients and modulates the expression of the Wolf-Hirschhorn syndrome candidate 1 gene (*WHSC1*, also known as multiple myeloma set domain [(*MMSET*)], which encodes a protein with homology to histone methyltransferases, and the receptor tyrosine kinase fibroblast growth

Recent studies using whole-genome or exome sequencing of MM tumors and their comparison to matched normal DNAs were performed. Several new and unexpected oncogenic mechanisms were suggested by the pattern of somatic mutation across the data set. These include the mutation of genes involved in protein translation (seen in nearly half of the patients), genes involved in histone methylation, and genes involved in blood coagulation. In addition, a broader than anticipated role of NF-κB signaling was indicated by mutations in 11 members of the NF-κB pathway. Of potential immediate clinical relevance, activating mutations of the kinase BRAF (proto-oncogene B-Raf) were observed in 4% of patients, suggesting the evaluation of BRAF inhibitors in

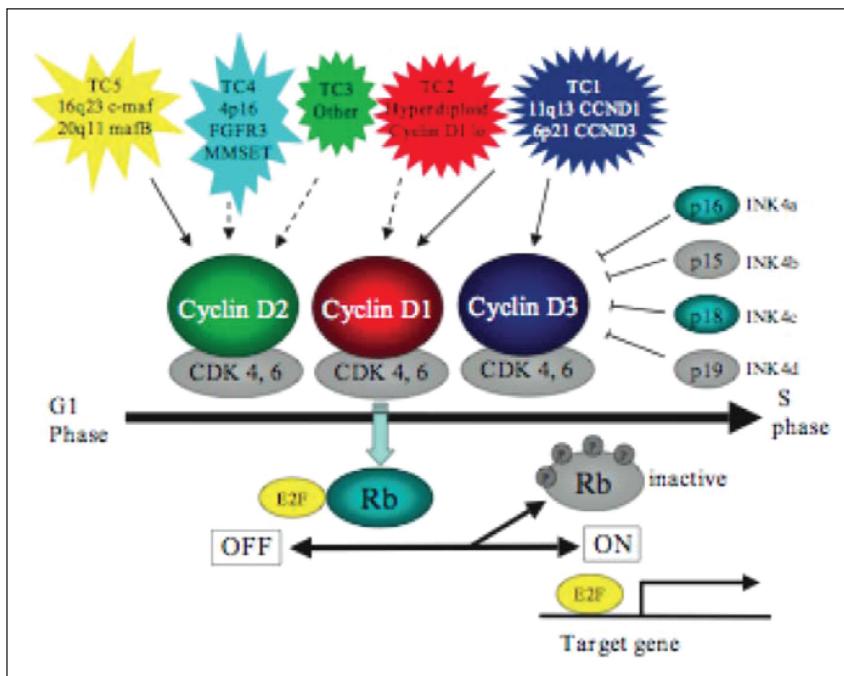


Figure 23-1 The critical role of cyclin D dysregulation in the pathogenesis of multiple myeloma (MM) highlights the importance of the cyclin D/Rb pathway and suggests that there may be a therapeutic opportunity in targeting this pathway for all molecular subtypes of MM.

MM clinical trials. Egan et al. (2012) recently studied a single patient with MM at four different times during her disease treatment and eventual progression to plasma cell leukemia. Whole-genome sequencing at each of these time points revealed distinct single nucleotide variants in 10 candidate genes that were present at all time points, indicating that these mutations may be essential for initial myelomagenesis. Several other genetic mutations, including those in *RBI* and *TP53*, were identified only at the plasma cell leukemia phase, however, suggesting that these candidate genes may be responsible for extramedullary transformation.

Classic genetics alone cannot explain the diversity of phenotypes within a population or the different susceptibilities to disease seen in twins. Epigenetics is defined as heritable changes in gene expression that do not involve a change in DNA sequence. Epigenetics have now been widely accepted as major regulators of tumor suppressors and oncogenes and in the development of many tumor types. The main epigenetics changes include DNA methylation and histone modification. MicroRNAs (miRNAs) also small RNA molecules that also are involved in the regulation of gene expression. Changes in methylation patterns, histone deacetylation, and miRNA regulation have been identified in MM and shown to play a critical role in the progression of this disease.

Role of the BM microenvironment in MM pathogenesis

Despite limits to our understanding of the molecular events of neoplastic transformation in MM, substantial advances have been made in understanding the biology of the disease

Table 23-2 Myeloma chromosomal alterations.

Chromosome anomalies: incidence

Conventional banding: 30%-50% of patients
 Interphase FISH: .90% of patients
 SKY: ? ~100%

Specific chromosome changes

14q32: majority of cases
 11q13: most common (bcl-1 locus, 30%)
 4p16 (FGFR3, MMSET, 25%)
 8q24 (c-myc, 5%)
 16q23 (c-maf, 1%)
 6p25 (Irf4, rare)
 13 deletion (Rb)

FISH = fluorescence in situ hybridization; SKY = spectral karyotyping.
 Bergsagel L, et al. Promiscuous translocations into immunoglobulin heavy chain switch regions in multiple myeloma. *Proc Natl Acad Sci USA*. 1996;93:13931-13936.

Facon T, Avet-Loiseau H, Guillerm G, et al. Chromosome 13 abnormalities identified by FISH analysis and serum b₂-microglobulin produce a powerful myeloma staging system for patients receiving high-dose therapy. *Blood*. 2001;97:1566.

Fonseca R, Bailey RJ, Ahmann GJ, et al. Genomic abnormalities in monoclonal gammopathy of undetermined significance. *Blood*. 2002;100:1417.

Kuehl WM, Bergsagel PL. Multiple myeloma: evolving genetic events and host interactions. *Nat Rev Cancer*. 2002;2:175.

Tricot G. *Br J Hematol* 2002;116:211.

through the study of the BM microenvironment, which appears to be fundamental for the proliferation, survival, and resistance of myeloma. The cellular components of the BM include BM stromal cells or mesenchymal cells (BMSCs),

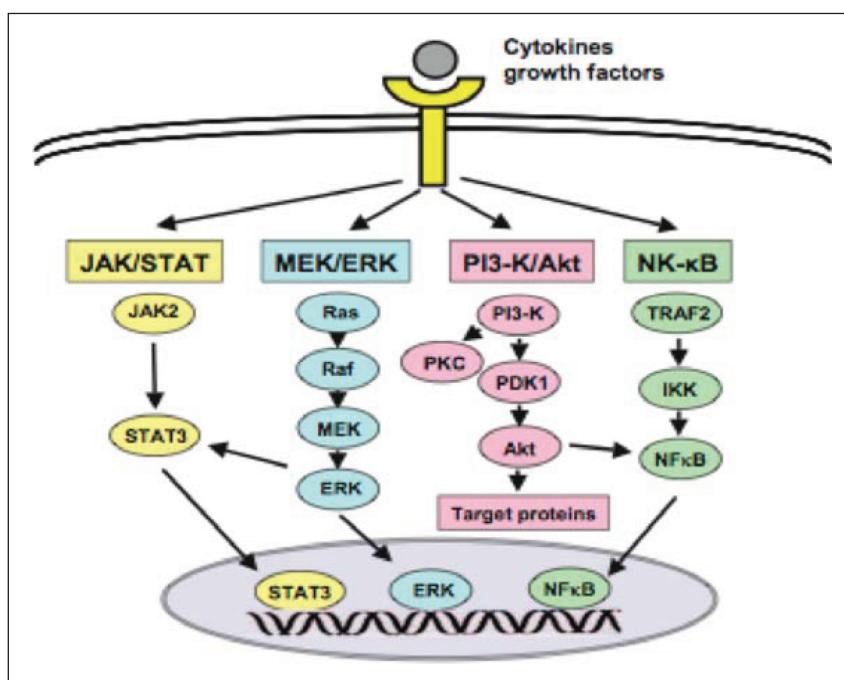


Figure 23-2 Cytokine-mediated signaling cascades in MM. IL-6 triggers Ras/Raf/MEK/ERK-mediated proliferation; induces JAK2/STAT3 signaling promoting MM cell survival and activates PI3K/Akt signaling, thereby mediating antiapoptosis and drug resistance in MM cells. IGF-1, VEGF, and SDF-1 α activate ERK and PI3K/Akt signaling cascades. TNF α triggers NF κ B activation, thereby enhancing MM cell-BMSC adherence and cytokine secretion.

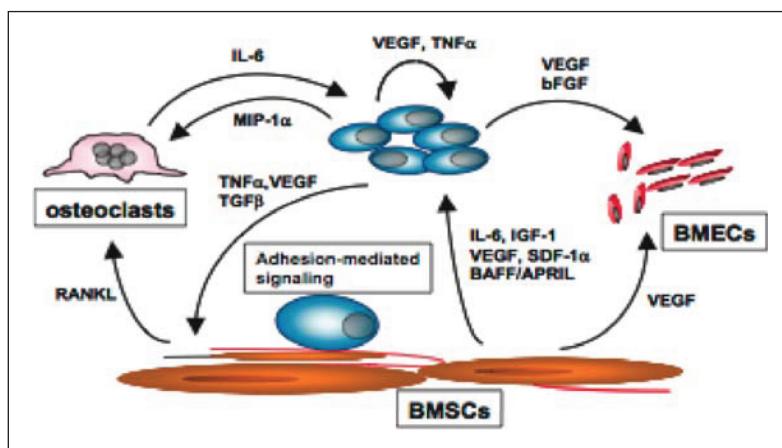


Figure 23-3 Growth factors for MM cells in the BM microenvironment. MM cells secrete VEGF, bFGF, TNF α , TGF β , and MIP-1 α . BMSCs secrete IL-6, VEGF, SDF-1 α , and RANKL. Cytokines secreted from either MM cells or BMSCs further augment these cytokine secretions. TNF α , VEGF, and TGF β from MM cells enhance IL-6 secretion from BMSCs. VEGF and bFGF trigger angiogenesis in BM endothelial cells. MIP-1 α and RANKL trigger OCL formation, thereby inducing bone destruction in MM. OCLs also produce IL-6, promoting MM cell growth and antiapoptosis.

osteoclasts (OCLs), osteoblasts (OBs), and vascular endothelial cells (BMECs). Cytokines, such as interleukin 6 (IL-6), play important roles in the pathogenesis and progression of the disease and its pathophysiologic manifestations. BMSCs are a major source of IL-6, which acts to promote myeloma cell survival by inhibiting apoptosis (Figure 23-2). IL-6 also contributes to bone loss in myeloma by stimulating OCL formation and inhibiting bone formation. It has been demonstrated that malignant plasma cells interact with extracellular matrix (ECM) proteins and that these interactions protect the cells from chemotherapy-induced and radiation therapy-induced cell death (Figure 23-3).

MM cells home to the BM and adhere to ECM proteins and to BMSCs, a process that not only localizes tumor cells in the BM milieu but also has important functional sequelae. Specifically, adhesion of MM cells to ECM proteins confers

cell adhesion-mediated drug resistance (CAM-DR), and binding of MM cells to BMSCs triggers transcription and secretion of cytokines (ie, IL-6, insulin-like growth factor-1 [IGF-1], or vascular endothelial growth factor [VEGF]) from BMSCs, which not only promotes growth, survival, and migration of MM cells but also further confers resistance to conventional chemotherapy.

Osteoclasts in MM

Osteolytic bone lesions develop in >70%-80% of patients throughout the axial skeleton and are one of the major sources of morbidity and mortality for patients with MM. These lesions are frequently associated with severe and debilitating bone pain, and pathologic fractures. The usual balance between bone resorption and new bone formation is

lost in many cases of MM, resulting in bone destruction and the development of osteolytic lesions. Bone destruction develops adjacent to MM cells, yet not in areas of normal BM. Furthermore, new bone formation, which normally would develop at sites of prior bone destruction, is absent and there is evidence, at these sites of apoptotic OBLs. The lack of OBLs explains why nuclear medicine bone scans underestimate the degree of bone destruction in patients with MM. The bone lesions that develop rarely heal, even when patients are in a complete remission. As a consequence of increased bone resorption, nearly 15% of MM patients develop hypercalcemia. The combination of hypercalcemia, pathologic fractures, nerve compression, and severe bone pain lead to significant morbidity and mortality for patients with MM.

There are several factors implicated in OCL activation, including receptor activator of NF- κ B ligand (RANKL), macrophage inflammatory protein-1a (MIP-1a), interleukin-3 (IL-3), and IL-6. RANKL is a member of the tumor necrosis factor (TNF) family and plays a major role in the increased osteoclastogenesis implicated in MM bone disease. MM cell binding to neighboring stromal cells within the BM of patients with MM results in increased RANKL expression. This leads to an increase in OCL activity through the binding of RANKL to its receptor, on OCL precursor cells, which further promotes their differentiation. RANKL is also involved in inhibition of OCL apoptosis.

Osteoblasts in MM

Several factors are felt to be responsible for suppression of OBL activity in MM and the precise mechanisms through which they execute their effect currently is under investigation. Some of these markers include: IL-3, Dickkopf- 1 (DKK1), secreted frizzled-related protein-2, and IL-7. Certain factors (DKK1 and sFRP-2) appear to affect the Wnt signaling pathway, critical for OBL differentiation, whereas others (IL-3 and IL-7) do not appear to directly affect this signaling pathway. These are soluble inhibitors of signaling pathways which affect OBL differentiation and not OBL survival, suggesting that their inhibitory effects should be reversible when MM cells are no longer present. DKK1 plays an important role in OBL suppression in myeloma. MM cells secrete DKK1, which inhibits OBL differentiation. A high correlation of DKK1 gene expression levels with the extent of bone disease was demonstrated in MM patients. A more recent study showed that levels of DKK1 were elevated in patients with MM as compared with either those with MGUS or normal controls. A sustained decrease in levels following autologous stem cell transplantation (ASCT) correlated with a normalization of markers of bone turnover, suggesting a return of OBL activity.

Diagnostic evaluation

Although some patients with MM are asymptomatic at the time of presentation, most patients present with symptoms. The most common presenting complaint is bone pain from lytic lesions or compression fractures, but patients also may be symptomatic from anemia, hypercalcemia, or renal insufficiency. Recurrent infections are frequent as a result of impaired cellular immunity and the reduced levels of normal Ig.

The initial evaluation of a suspected monoclonal gammopathy should include both serum and urine protein electrophoresis with immunofixation to identify and quantify the M protein (Table 23-3). The majority of patients will have a detectable M protein, but approximately 1%-3% can present with a nonsecretory myeloma that does not produce light or heavy chains. True nonsecretory myeloma is thus rare, not least because, with the availability of serum FLC testing, it is recognized that M protein is present. The most common M protein is IgG, followed by IgA and light-chain-only disease. IgD and IgE are relatively uncommon and can be more difficult to diagnose because their M spikes often are very small. Up to 20% of patients will produce only light chains, which may not be detectable in the serum because they pass through the glomeruli and are excreted in the urine. The standard evaluation of a documented monoclonal gammopathy includes a complete blood count with differential, calcium, serum urea nitrogen, and creatinine. As mentioned, serum FLC testing is also a useful diagnostic test. Bone disease is best assessed by skeletal survey. Bone scans are not a sensitive measure of myelomatous bone lesions because the radioisotope is poorly taken up by lytic lesions in MM, as a result of OBL inhibition. Magnetic resonance imaging (MRI) is useful for the evaluation of solitary plas-

Table 23-3 Monoclonal gammopathy: staging studies.

Complete blood count, including differential to assess for circulatory plasma cells
Chemistry with BUN, creatinine
Serum protein electrophoresis with immunofixation
Quantitative immunoglobulins
24-hour urine immunoelectrophoresis
Skeletal survey (plain films)
Serum B2-microglobulin, albumin
Bone marrow aspirate and biopsy
Cytogenetics/FISH
Serum free light chain
MRI*
PET scan*

*MRI and PET scans are used in specific circumstances and are not routinely performed in all patients with monoclonal gammopathies. BUN = blood urea nitrogen; FISH = fluorescence in situ hybridization; MRI = magnetic resonance imaging; PET = positron emission tomography.

macytoma of bone and for the evaluation of paraspinal and epidural components. 18F-FDG positron emission tomography (PET)/computed tomography (CT) scans are more sensitive in the detection of active lesions in the whole body. PET and MRI sometimes may identify otherwise-cryptic sites of disease that are not seen on a skeletal survey and thus may distinguish those patients who actually have systemic myeloma rather than an isolated lesion of solitary plasmacytoma.

A BM aspiration and biopsy is important to quantify the plasma cell infiltrate and adds important prognostic information with cytogenetic evaluation, including FISH.

Staging and prognostic factors

The criteria for the diagnosis of MM, SMM, and MGUS are detailed in Table 23-1. Distinction among these entities is important for making treatment decisions and prognostic recommendations.

Several staging systems exist. The most widely used myeloma staging system since 1975 has been the Durie-Salmon, in which the clinical stage of disease is based on several measurements, including levels of M protein, serum hemoglobin value, serum calcium level, and the number of bone lesions. The International Staging System (ISS), developed by the International Myeloma Working Group (IMWG), now also is used widely. Both systems are outlined in Table 23-4. This validated ISS is based on two prognostic factors, serum levels of β_2 M and albumin, and is composed of three stages: β_2 M \leq 3.5 mg/dL and albumin \geq 3.5 g/dL (median survival, 62 months; stage I); β_2 M $<$ 3.5 mg/dL and albumin $<$ 3.5 g/dL or β_2 M \geq 3.5 to $<$ 5.5 mg/dL (median survival, 44 months; stage II); and β_2 M \geq 5.5 mg/dL (median survival, 29 months; stage III). With an increased understanding of the biology of myeloma, other factors have been shown to correlate well with clinical outcome and now commonly are used. Cytogenetic abnormalities as detected by FISH techniques have been shown to identify patient populations with

very different outcomes. Loss of the long arm of chromosome 13 is found in up to 50% of patients and, when detected by metaphase chromosome analysis, is associated with poor prognosis, as is a hypodiploid karyotype. t(4;14) and -17p13.1 typically are associated with poor outcome, whereas the t(11;14) and hypodiploidy are associated with improved survival.

Survival

The survival times of MM patients have improved in the last decade. A recent study examined the outcome of two groups of patients cared for at Mayo Clinic, one from the time of diagnosis and the other from the time of relapse, to examine survival trends over time. Among 387 patients who relapsed after SCT, a clear improvement in overall survival from the time of relapse was seen, with those relapsing after 2000 having a median overall survival of 23.9 versus 11.8 months ($P < .001$) for those who relapsed before this date. Patients treated with one or more of the newer drugs (thalidomide, lenalidomide, bortezomib) had longer survival from relapse (30.9 vs. 14.8 months; $P < .001$). In a larger group of 2,981 patients with newly diagnosed myeloma, those diagnosed in the past decade had a 50% improvement in overall survival (44.8 vs. 29.9 months; $P < .001$).

Another study estimated trends in age-specific 5- and 10-year relative survival of patients with MM in the United States from 1990-1992 and from 2002-2004 from the 1973-2004 database of the Surveillance, Epidemiology, and End Results (SEER) Program. Overall, the 5-year survival rate increased from 28.8% to 34.7% ($P < .001$), and 10-year survival increased from 11.1% to 17.4% ($P < .001$) between 1990-1992 and 2002-2004. The most significant increases were seen in patients $<$ 50 years old, with 5- and 10-year relative survival rates of 56.7% and 41.3% in 2002-2004, and in those patients 50-59 years old, for which 5- and 10-year relative survival of 48.2% and 28.6% were observed. By contrast, only moderate improvement was seen in the age-group

Table 23-4 Staging systems for multiple myeloma: Durie-Salmon staging system and International Staging System (ISS).

Stage	Durie-Salmon system	ISS staging system
I	Calcium normal or $<$ 12 mg/dL, hemoglobin value $>$ 10 g/dL, normal skeletal survey or solitary plasmacytoma, low M protein with an IgG $<$ 5 g/dL or IgA $<$ 3 g/dL, Bence Jones protein $>$ 4 g/24 hours	β_2 M \leq 3.5 and albumin \geq 3.5 g/dL
II	Neither stage I nor stage III	β_2 M $>$ 3.5 but $<$ 5.5, or albumin $>$ 3.5 g/dL
III	One of the following: hemoglobin value $<$ 8.5 g/dL, calcium $>$ 12 mg/dL, multiple lytic lesions, high M component with an IgG $>$ 7 g/dL or IgA $>$ 5 g/dL, Bence Jones protein $>$ 12 g/24 hours	β_2 M \geq 5.5

*Durie-Salmon classification of A or B with A having normal renal function (creatinine, 2.0 mg/dL) and B having abnormal renal function (creatinine, 2.0 mg/dL).

β_2 M = β_2 microglobulin; Ig = immunoglobulin; IL = interleukin.

60-69 years, and essentially no improvement was achieved among older patients.

Treatment

Patients with MGUS should have repeat protein studies approximately every 6 months for 2-3 years to assess possible progression and annually thereafter. Patients with SMM should be followed approximately every 3 months with serum protein electrophoresis, blood counts, and creatinine, and with skeletal survey every 12 months or sooner if new symptoms develop. Individuals with progressive disease should be considered for treatment. Evidence of progressive disease includes increasing M protein, declining hemoglobin, increased creatinine, lytic lesions, or recurrent infections related to depressed levels of normal Ig.

Patients should not receive treatment unless they have symptomatic MM. The criteria used to determine initiation of therapy are the CRAB criteria defined as calcium elevation (>11.5 g/dL), renal insufficiency (creatinine >2 mg/dL), anemia (hemoglobin <10 g/dL or 2 g $<$ normal, and bone disease (lytic or osteopenic). Other examples of active disease include repeated infections, secondary AL, hyperviscosity, or hypogammaglobulinemia.

Several studies are ongoing to examine the role of treatment of patients with high-risk SMM, who are likely to have a 5-year progression rates of 76%. Musto et al. (2008) conducted a prospective, open-label, randomized phase III trial comparing the administration of zoledronic acid vs. observation in 163 SMM patients. Monthly zolderonic acid for 1 year in SMM patients reduced the development of skeletal-related events (SREs) at the time of progression (55.5% vs. 78.3%, $P = .41$) but did not affect the TTP of symptomatic disease. Several studies, including agents such as thalidomide, previously were examined in SMM but were too small to adequately answer the question of early therapeutic interventions. It is noteworthy that all these trials included all patients with SMM and did not select a high-risk group. In the recently concluded phase III Program for the Study and Treatment of Hematological Malignancies, Spanish Society of Hematology (PETHEMA) trial, high-risk SMM patients were randomized to receive lenalidomide and dexamethasone as induction followed by lenalidomide as maintenance versus no treatment alone. High-risk was defined as the presence of both $>10\%$ PC and >3 g/dL M-protein, or if the patients had only one criterion, then they also were required to have $>95\%$ aPC plus immunoparesis. Patients randomized to the treatment arm received nine cycles of Len/Dex followed by maintenance Len. After a median follow up of 32 months, 9 (15%) patients in the treatment arm and 37 (59%) patients in the observation arm had progressed (hazard ratio [HR], 6.0; 95% confidence interval [CI] 2.9-12.6; $p < .0001$). The estimated 3-year overall survival was 93% in the treatment arm

and 76% in the observation arm ($P = .04$). Larger trials are ongoing and are needed before treatment in the absence of CRAB criteria is considered standard.

Frontline therapy for symptomatic MM includes either conventional chemotherapy or high-dose chemotherapy (HDT) supported by autologous or allogeneic SCT, depending on patient characteristics, such as performance status, age, availability of a sibling donor, comorbidities, and, in some cases, patient and physician preferences. Response to therapy is commonly measured by a reduction in M protein levels in serum or urine and the reduction in size or disappearance of plasmacytomas. The international uniform response criteria for MM have expanded on the European Group for Blood and Marrow Transplantation (EBMT) criteria to provide a more comprehensive evaluation system. Despite high response rates to frontline therapy, virtually all patients eventually relapse. Thus, research efforts are concentrated on improving frontline therapy to enhance and prolong response, reduce the rate of relapse, and improve the efficacy of treatment at relapse. Importantly, achievement of response has been associated with improved survival in SCT trials with high-dose therapy. Similarly, TTP has been shown to be an important surrogate for improved survival. Tables 23-5 and 23-6 show the international uniform response criteria for MM.

Initial therapy for patients eligible for SCT

Patients with newly diagnosed myeloma who are considered eligible for HDT and SCT should avoid alkylators such as melphalan, which can interfere with adequate stem cell mobilization, regardless of whether an early or delayed transplantation is contemplated. Several new combinations have improved the response and survival of patients with MM. The introduction of targeted therapeutic agents, such as thalidomide, bortezomib, and lenalidomide, has revolutionized options of treatment in patients with MM. These novel agents, in combination with dexamethasone or cytotoxic therapies, have shown better responses compared with traditional agents, such as single-agent dexamethasone, or conventional chemotherapy, such as high-dose dexamethasone or doxorubicin, vincristine, and intermittent high-dose dexamethasone (VAD).

Goals of therapy

There is no question that the survival of MM patients has improved significantly in the last decade. Survival for an individual patient with MM varies according to age, stage, and performance status, and molecular prognostic factors. It is important to keep these variables in mind when interpreting clinical trial results. Whenever possible, changes in what is considered standard therapy should be based on improved overall survival (OS) and quality of life.

Table 23-5 Response category.

CR	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and ≤5% plasma cells in bone marrow
sCR	CR as described above, plus: normal free light chain (FLC) ratio and absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence
VGPR	Serum and urine M-protein detectable by immunofluorescence but not on electrophoresis or 90% or greater reduction in serum M-protein plus urine M-protein level <100 mg per 24 hours
PR	≥50% reduction of serum M-protein and reduction in 24-h urinary M-protein by ≥90% or to <200 mg per 24 h If the serum and urine M-protein are unmeasurable, ≥50% decrease in the difference between involved and unininvolved FLC levels is required in place of the M-protein criteria If serum and urine M-protein are unmeasurable, and serum free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was ≥30%
	In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size of soft tissue plasmacytomas is also required
SD	Not meeting criteria for CR, VGPR, PR, or progressive disease

Risk stratification and choice of therapy

Risk stratification in patients with MM is based on their clinical characteristics as well as their molecular characteristics, such as poor cytogenetic features. Patients with adverse cytogenetics or aggressive clinical features, such as extramedullary disease (EMD), short duration of response to prior therapy, or renal failure, are classified as having high-risk disease. Those with indolent clinical features, prolonged response to therapy, and absence of adverse cytogenetics are classified as having standard-risk relapsed MM. Clinical trial participation is an important option for all patients with MM whether high risk or standard risk.

Patients with newly diagnosed myeloma who are considered eligible for HDT and SCT should avoid alkylators, such as melphalan, which can interfere with adequate stem cell mobilization, regardless of whether an early or delayed transplantation is contemplated.

Immunomodulators in newly diagnosed transplantation-eligible patients

Thalidomide

Several phase III clinical trials have shown that the combination of thalidomide and dexamethasone (TD) is superior to dexamethasone alone in induction therapy in transplantation-eligible patients with newly diagnosed MM. Rajkumar et al.

Table 23-6 Relapse subcategory.

Progressive disease	any one or more of the following: Increase of ≥25% from baseline in: <ul style="list-style-type: none"> - Serum M-component and/or (the absolute increase must be ≥0.5 g/dL) - Urine M-component and/or (the absolute increase must be ≥200 mg/24 h) - Only in patients without measurable serum and urine M-protein levels: the difference between involved and unininvolved FLC levels. The absolute increase must be ≥10 mg/dL - Bone marrow plasma cell percentage: the absolute percentage must be ≥10% - Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas - Development of hypercalcemia (corrected serum calcium >11.5 mg/dL or 2.65 mmol/L) that can be attributed solely to the plasma cell proliferative disorder
Clinical relapse	one or more of the following: <ul style="list-style-type: none"> - Development of new soft tissue plasmacytomas or bone lesions - Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by the sum of the products of the cross-diameters of the measurable lesion - Hypercalcemia (≥11.5 mg/dL) [2.65 mmol/L] - Decrease in hemoglobin of >2 g/dL [1.25 mmol/L] (see Table 23-3 for further details) - Rise in serum creatinine by 2 mg/dL or more [177 mmol/L or more]
Relapse from CR	one or more of the following: <ul style="list-style-type: none"> - Reappearance of serum or urine M-protein by immunofixation or electrophoresis - Development of ≥5% plasma cells in the bone marrow - Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcemia)

(2006) randomized 207 individuals with newly diagnosed MM to either TD or dexamethasone; TD included thalidomide 200 mg daily and dexamethasone 40 mg on days 1-4, 9-12, and 17-20. The combination of TD showed a superior overall response rate (ORR; 63% vs. 41%) and complete response (CR) rate (4% vs. 0%) compared with the dexamethasone arm. In a second phase III trial of TD versus dexamethasone in 470 transplantation-eligible MM patients, the combination of TD showed an ORR of 64%, whereas dexamethasone alone produced an ORR of 46%. TTP was significantly longer in patients who received the combination (22.6 vs. 6.5 months). Another study by Macro et al. (2006) randomized 203 patients with previously untreated MM to either four cycles of TD or three cycles of VAD induction, followed by high-dose melphalan 200 mg/m² and ASCT. The rate of very good partial response (VGPR) or better was higher in the TD group before stem cell collection (24.7% vs. 7.3%) compared with the group receiving VAD chemotherapy.

The most common adverse effects (AEs) of thalidomide include deep vein thrombosis, rash, neuropathy, sedation, fatigue, and constipation. Peripheral neuropathy (PN) caused by axonal injury and loss of large-diameter myelinated nerve fibers is dose and time dependent. Venous thromboembolism (VTE) occurred in 2%-15% of patients with relapsed MM receiving thalidomide and either dexamethasone or chemotherapy. Other less common but important toxicities include bradycardia, hypothyroidism, and rash.

Lenalidomide

A phase II trial of lenalidomide 25 mg on days 1-21 and dexamethasone 40 mg on days 1-4, 9-12, and 17-20 of each 28-day cycle showed an ORR rate of 91%, with partial response (PR), VGPR, and CR rates of 35%, 38%, and 18%, respectively. In the phase III Eastern Cooperative Oncology Group (ECOG) E4A03 trial, 445 patients with newly diagnosed MM were randomized to receive lenalidomide 25 mg on days 1-21 and either high-dose dexamethasone (RD; 40 mg daily on days 1-4, 9-12, and 17-20) or low-dose dexamethasone (Rd; 40 mg on days 1, 8, 15, and 22). There was a higher degree of grade 3 toxicity that occurred in 50% of RD-treated patients as opposed to 30% of patients who received RD. RD yielded superior OS rates compared with RD (96% vs. 88% at 1 year and 87% vs. 75% at 2 years, respectively). However, RD was superior to RD in the ORR (82% vs. 70%, respectively). This study led to a significant change in the dosing of dexamethasone in all subsequent trials and in clinical care.

Another phase III trial of lenalidomide and dexamethasone compared with placebo/dexamethasone was conducted in 198 patients (Southwest Oncology Group [SWOG] trial S0232). In arm A, lenalidomide was given at 25 mg/d (28 of

35 days for three induction cycles, then 21 of 28 days as maintenance thereafter) plus high-dose dexamethasone (HD; 40 mg on days 1-4, 9-12, and 17-20 induction, then days 1-4 and 15-18 maintenance); in arm B, HD (same induction and maintenance schedules) was given plus placebo. One-year progression-free survival (PFS), ORR, and VGPR rate were superior with LEN-DEX (78% vs. 52%, $P = .002$; 78% vs. 48%, $P < .001$; 63% vs. 16%, $P < .001$), whereas 1-year overall survival was similar (94% vs. 88%; $P = .25$). The ORR (partial response or better) was 78% for LEN-DEX arm versus 48% for the Dex arm ($P < .0001$).

Myelosuppression and vascular thrombotic events are the most common toxicities observed with lenalidomide, and thromboprophylaxis is necessary in patients receiving lenalidomide alone or in combination. Unlike thalidomide, it is not associated with significant neuropathy. A macular rash occurs in up to 30% of patients who receive lenalidomide, but in most instances is mild and resolves with either topical corticosteroid therapy or brief interruption of therapy. Dose adjustment is necessary in patients with impaired renal function to avoid drug-related side effects. Concern has been raised regarding the impact of lenalidomide on stem cell collection before ASCT. A decrease in the total number of CD34 stem cells collected has been observed in patients mobilized with granulocyte colony-stimulating factor (G-CSF) after induction with lenalidomide plus dexamethasone as compared with induction with VAD, TD, or dexamethasone alone. This appears to be related to the duration of lenalidomide therapy as well as the age of the patient. For this reason, early mobilization of stem cells, preferably within the first four cycles of initial therapy, is now recommended.

Proteasome inhibitors in newly diagnosed transplantation-eligible patients

Bortezomib

The French Myeloma Intergroup (IFM) randomized 480 newly diagnosed MM patients to induction therapy with VAD or bortezomib plus dexamethasone (VD). A second randomization was then performed to either receive or not receive two cycles of dexamethasone, cyclophosphamide, etoposide, and cisplatin (DCEP) consolidation before ASCT. VD was superior to VAD induction with respect to rates of VGPR or better (46.7% vs. 18.6%) and CR/near CR (nCR; 21.3 vs. 8.3%). Clinical benefit associated with VD persisted after ASCT with respect to rates of VGPR or better (40.8% vs. 28.8%) and CR/nCR (71.8% vs. 51%). Response rates were not improved in either treatment group by DCEP consolidation. Another phase III study randomized 883 transplantation-eligible patients to either VAD (Arm A) or bortezomib plus doxorubicin and dexamethasone (PAD) (Arm B) followed by stem cell

mobilization and either single or tandem ASCT. Patients in the VAD group then received maintenance therapy with thalidomide 50 mg daily, whereas patients in the PAD arm received bortezomib 1.3 mg/m² every other week as maintenance. In a preliminary analysis, PAD induction was superior to VAD with respect to ORR (80% vs. 64%), rate of VGPR or better (41% vs. 17%), and CR rate (5% vs. 0%). The benefit of PAD also was observed after ASCT, with superior ORR (92% vs. 77%) and CR rate (15% vs. 4%). In the PAD arm, bortezomib maintenance deepened responses further, with an increase in the CR/nCR rate from 23% to 35%. A follow-up report of this study indicates patients treated on Arm B (bortezomib containing regimen) had improved OS. A surprising finding was that patients with del(17p13) benefited the most from the bortezomib-containing treatment with improved PFS (12.0 months vs. 26.2 months) and 3-year OS (17% vs. 69%).

The main AEs of bortezomib are peripheral neuropathy, thrombocytopenia, and gastrointestinal symptoms and herpes zoster reactivation. Bortezomib-associated PN is cumulative and dose related, and symptom-guided dose modification based on a standard dose reduction algorithm is recommended. A phase III trial was done that compared bortezomib administered intravenously to subcutaneously. The trial included 222 patients with relapsed myeloma. Response rates and survival were not significantly different. Peripheral neuropathy was significantly less common with subcutaneous than with intravenous administration. The administration of weekly Bortezomib instead of twice a week also has reduced the risk of neuropathy. Thrombocytopenia is usually cyclic, with a decline followed by recovery during the period off therapy. Because of the risk of herpes zoster reactivation with Bortezomib, antiviral prophylaxis is recommended for all patients receiving bortezomib. Rare instances of lung injury, including bronchiolitis obliterans with organizing pneumonia and pulmonary fibrosis, have been reported.

Combination of these agents in newly diagnosed transplantation-eligible patients

The PETHEMA group compared safety and efficacy of TD and bortezomib, thalidomide, and dexamethasone (VTD) to VBMC/VBAD/V (vincristine, bis-chloroethylnitrosourea [BCNU], cyclophosphamide, melphalan, prednisone/vincristine, BCNU, doxorubicin, dexamethasone/bortezomib) in newly diagnosed symptomatic MM patients. Patients were randomized to VBMC/VBAD (four cycles) plus bortezomib (two cycles), TD (six cycles), or VTD prior to high-dose MEL200 conditioned ASCT. Following SCT, patients underwent a second randomization to interferon- α 2b, thalidomide, or thalidomide plus bortezomib maintenance. ORR and PFS were significantly better with VTD; however, this did not translate into a significant difference in OS. A phase III

study randomized 480 transplantation-eligible patients with newly diagnosed MM to VTD or TD. Preliminary analysis has demonstrated the superiority of VTD compared with TD with respect to ORR (93% vs. 9%), CR/nCR rate (31% vs. 11%), and rate of VGPR or better (62% vs. 28%). No data regarding whether this translated to improved OS were published, however. Bortezomib in combination with lenalidomide and dexamethasone (VRD) has been proven to be effective in newly diagnosed myeloma. Sixty-six patients were enrolled in a phase I/II trial and received bortezomib 1.0 or 1.3 mg/m² (days 1, 4, 8, 11), lenalidomide 15–25 mg (days 1–14), and dexamethasone 40 or 20 mg (days 1, 2, 4, 5, 8, 9, 11, 12). The ORR (PR or better) was 100%. The most common toxicity was sensory neuropathy, seen in 80%. Phase III studies are ongoing with the use of RVD combination. A randomized phase II trial evaluated combinations of bortezomib (V) and dexamethasone (D) with either lenalidomide (R) or cyclophosphamide (C) in previously untreated MM. Patients received V 1.3 mg/m² (days 1, 4, 8, 11) and D 40 mg (days 1, 8, 15), with either C 500 mg/m² (days 1, 8) and R 15 mg (days 1–14; VDCR), R 25 mg (days 1–14; VDR), C 500 mg/m² (days 1, 8; VDC), or C 500 mg/m² (days 1, 8, 15; VDC-mod) in 3-week cycles (maximum eight cycles), followed by maintenance with V 1.3 mg/m² (days 1, 8, 15, 22) for four 6-week cycles. Responses of VGPR or better were seen in 58%, 51%, 41%, and 53% of patients (VDCR, VDR, VCD, and VCD-mod, respectively); the corresponding 1-year PFS was 86%, 83%, 93%, and 100%, respectively. No advantage was noted with VDCR over the three-drug combinations.

Proteasome inhibitors: bortezomib

In a phase III trial, 682 ASCT-ineligible patients with previously untreated MM were randomized to either bortezomib plus melphalan and prednisone (VMP) or MP alone. All patients received melphalan 9 mg/m² and prednisone 60 mg/m² on days 1–4 of each 6-week cycle, whereas bortezomib was administered in the combination arm at 1.3 mg/m² on days 1, 4, 8, 11, 22, 25, 29, and 32 during cycles 1–4 and on days 1, 8, 22, and 29 during cycles 5–9. VMP was superior to MP in terms of the study's primary endpoint of TTP (24 vs. 16.6 months), as well as secondary endpoints of CR rate (30% vs. 4%) and duration of response (19.9 vs. 13.1 months).

Intensification therapy

ASCT is the most widely used transplant in MM. Although not curative, autologous HDT/SCT improves CR rates and prolongs median OS in myeloma by ~12 months, with a low treatment-related mortality (reported as <5%, and in most institutions <1%). Melphalan 200 mg/m² is the most widely used preparative regimen. There has been only one prospective

randomized controlled trial comparing conditioning regimens in patients with myeloma. The IFM randomized 282 patients to receive either melphalan (140 mg/m²) plus total-body irradiation or melphalan alone (200 mg/m²). There was no difference in response rates or event-free survival (EFS). Survival at 45 months favored the melphalan-alone arm (65.8 vs. 45.5%, $P=.05$). The first phase III trial in more than 200 patients was reported by Attal et al. (1996) and demonstrated a survival benefit for HDT/SCT compared with standard chemotherapy, with an EFS rate at 5 years of 28% versus 10% in the high-dose versus conventional-dose groups, respectively. The Medical Research Council VII trial was the second published study addressing the question of standard chemotherapy versus HDT/SCT. This trial included 401 randomized patients. The trial found that the CR rates (8% vs. 44%), the median EFS (19 vs. 31 months), and the OS (42 vs. 54 months) all significantly favored the HDT/SCT arm. Another randomized trial (the PETHEMA study) of intensified therapy plus autologous transplantation compared with intensified therapy alone did not demonstrate a survival benefit with the transplantation arm. This trial was dissimilar from the other randomized trials in that only those patients who responded to therapy were randomized, possibly suggesting that patients with an excellent response to the induction therapy given do not require consolidation with HDT/SCT.

Although HDT/SCT prolongs survival in younger patients with myeloma, its timing (early vs. delayed) is an area of debate. Early transplantation is more typical than delayed, largely due to improvement in quality of life among patients getting early transplant. Given effective new agents to treat myeloma currently are available, some patients and physicians may choose to delay transplantation. Large phase III clinical trials are being performed to examine this question further as novel therapies are integrated into initial therapy and as maintenance.

The concept of double or tandem transplants was originated by Barlogie and colleagues at the University of Arkansas. With tandem HDT/SCT, patients receive a second planned HDT/SCT after recovery from the first procedure. The IFM 94 randomized trial found significantly better EFS and OS in recipients of double versus single HDT/SCT, but only in a subset of patients. Survival advantage from the tandem approach primarily was limited to patients achieving PR or less with the first SCT and with good prognostic factors. Conversely, patients achieving CR with the first SCT and patients with poorer prognostic factors (eg, chromosome 13 deletion and high β_2 M at presentation) did not appear to benefit from the second intensification. A similar benefit also was demonstrated in a randomized trial conducted in Italy; two other randomized trials are yet to show significant improvement in OS with tandem ASCT, but they

have shorter follow-up. In both the French and Italian trials, the benefit of a second HDT/SCT was restricted to patients who failed to achieve a CR or VGPR (>90% reduction in M protein level) with the first procedure. A meta-analysis was done comparing single transplant to tandem transplant for patients with myeloma and included 1,803 patients enrolled in six randomized controlled trials. Patients treated with tandem transplant did not have a better OS or EFS than patients treated with a single transplant.

The advantages of allogeneic HDT/SCT include lack of graft contamination with tumor cells and presence of a graft-versus-myeloma effect. Only 5%-10% of patients are candidates, however, because of age, availability of a human leukocyte antigen (HLA)-matched sibling donor, and adequate organ function. Furthermore, the high treatment-related mortality, mainly related to graft-versus-host disease (GVHD), has made conventional allogeneic transplantations unacceptable for most patients with myeloma. There are no prospective randomized controlled trials that examine the role of myeloablative allogeneic transplant in myeloma. The U.S. Intergroup trial S9321 attempted to answer this question. Newly diagnosed patients were treated with four cycles of chemotherapy. Patients were randomly assigned to either HDT/SCT or to resume standard dose chemotherapy. Patients who were <55 years of age with an HLA-compatible sibling donor were offered the option of allogeneic transplantation. The allogeneic arm was closed when an excessive first-year treatment-related mortality rate of 53% was observed. With 7 years of follow-up, the OS of the conventional chemotherapy, autologous transplant, and allogeneic transplant groups were identical at 39%. The allogeneic group showed a survival plateau, whereas the other two groups did not, suggesting that a portion of the allogeneic group were long-term survivors.

Another strategy is to use ASCT to cytoreduce the myeloma followed by a reduced-intensity conditioning allogeneic transplant. Four prospective trials have looked at this approach. Only one of the four trials noted improved overall survival in patients undergoing tandem autologous/allogeneic transplants. Given the toxicity of this approach and the lack of suitable donors, allogeneic transplant, whether myeloablative or RIC, should be considered experimental in patients with myeloma.

Maintenance therapy

Thalidomide maintenance

Maintenance therapy remains controversial in MM, and further studies are needed. One of the most significant studies tested thalidomide in a randomized phase III trial from the IFM; the IFM-99 02 trial assessed the impact of thalidomide

maintenance on duration of response after SCT in 780 patients. At 29 months of median follow-up from randomization (2 months after the second transplantation), patients randomized to thalidomide had improvement in EFS compared with patients randomized to no treatment or to pamidronate alone (arm 3 vs. arm 1 vs. arm 2, 52% vs. 36% vs. 37%; $P = .002$). Another study published by Barlogie et al. (2006) using thalidomide as maintenance in combination with dexamethasone and interferon after tandem transplantations showed no survival advantage with the addition of thalidomide. This was because the OS after relapse or progression was significantly lower in the thalidomide-treated patients ($P = .001$). The National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) conducted the MY.10 trial, which compared maintenance therapy with thalidomide (200 mg daily) and prednisone (50 mg every other day) to observation after ASCT. The study randomized 332 MM patients who had an ASCT within 1 year of initial treatment. With a median follow-up of 4 years, median PFS was significantly better with thalidomide and prednisone but maintenance with TP did not significantly prolong OS. Therefore, the role of thalidomide as maintenance therapy remains to be defined.

Lenalidomide maintenance

Lenalidomide maintenance has been studied in two randomized trials in the posttransplant setting. In addition one trial has looked at lenalidomide maintenance following induction chemotherapy. The IFM randomly assigned 614 patients <65 years of age who had nonprogressive disease after first-line transplantation to maintenance treatment with either lenalidomide (10 mg per day for the first 3 months, increased to 15 mg if tolerated) or placebo until relapse. The Cancer and Leukemia Group B (CALGB) assigned 460 patients who were <71 years of age and had stable disease or better following HDT/SCT to maintenance lenalidomide or placebo. Lenalidomide maintenance therapy improved median PFS in both studies. The IFM study reported no difference in OS. In the CALGB trial, overall survival at 3 years was 88% among patients in the lenalidomide group and 80% among patients in the placebo group. Both trials showed an increase in second primary malignancies in the group that received lenalidomide (8% vs. 3% in the CALGB trial and 3.1 per 100 patient-years in the lenalidomide group versus 1.2 per 100 patient-years in the placebo group in the IFM study).

The Italian group conducted a trial among patients ineligible for transplant. They randomly assigned patients to receive melphalan-prednisone-lenalidomide induction followed by lenalidomide maintenance (MPR-R; nine 4-week cycles of MPR followed by lenalidomide maintenance therapy until a relapse or disease progression occurred; 152 patients) or to receive MPR (153 patients) or MP (154 patients) without

maintenance therapy. MPR-R significantly prolonged PFS (median, 31 months vs. 14 months and 13 months). The 3-year OS rate was 70% with MPR-R, 62% with MPR, and 66% with MP. The 3-year rate of invasive second primary tumors was 7% with MPR-R, 7% with MPR, and 3% with MP. Although improved PFS was seen in all three trials, improved OS was seen in only one of the three trials and higher rates of second malignancies were seen in the lenalidomide maintenance group in all three trials. More data regarding long-term efficacy and safety is needed before we can conclude that maintenance lenalidomide is the standard of care.

Bortezomib maintenance

Bortezomib has been used in maintenance therapy after SCT in the HOVON-65/GMMG-HD4 study. Patients were randomly assigned to three cycles of standard VAD (arm A) or PAD (arm B). Patients received one (HOVON) or two (GMMG) high-dose melphalan (HDM) 200 mg/m² with ASCT. Maintenance consisted of thalidomide (T) 50 mg daily (arm A) or B 1.3 mg/m² twice weekly (arm B) for 2 years. The response rates were significantly higher on the PAD arm. In addition, patients treated with bortezomib had a better OS, indicating that bortezomib maintenance may be efficacious in MM. Because arm A never received bortezomib, however, it is difficult to assess whether the survival advantage seen in arm B was due to the bortezomib received during induction or maintenance. A further analysis showed, patients with del(17p13) benefited the most from the bortezomib-containing treatment. Among patients with del(17p13), the 3 year-OS for arm A was 17% and for arm B it was 69% ($P = .028$), suggesting a benefit for long-term administration of bortezomib.

Induction therapy for non-transplantation-eligible patients

Immunomodulators

Thalidomide

Several phase III trials have shown that thalidomide in combination with melphalan and prednisone (MPT) is an effective regimen for patients with newly diagnosed MM who are ineligible for ASCT. In one randomized phase III trial by Palumbo (2006), MPT was compared with melphalan and prednisone (MP) in 255 previously untreated individuals 60 years of age or older. The ORR and nCR/CR rate among patients who received MPT were 76% and 27.9%, respectively, compared with 47.6% and 7.2%, respectively, in the MP group. In another phase III trial by Facon et al. (2007), 447 individuals between the ages of 65 and 75 years with previously untreated MM were randomly assigned to receive

MP, MPT, or two courses of VAD followed by reduced-intensity ASCT using melphalan 100 mg/m². A PR or better was achieved in 35% of patients treated with MP, 76% of those treated with MPT, and 65% of those who received VAD followed by melphalan-ASCT. CR rates were 2%, 13%, and 18% in the MP, MPT, and VAD followed by melphalan-ASCT arms, respectively. Although response rates in the MPT and melphalan-ASCT arms were similar, MPT produced superior PFS (27.5 vs. 19.4 months) and median OS (51.6 vs. 38.3 months).

A trial was done that randomly assigned 289 elderly patients to treatment with thalidomide dexamethasone or MP. TD resulted in a higher proportion of complete and very good remissions (26% vs. 13%; $P=.006$) and overall responses (68% vs. 50%; $P=.002$) compared with MP, but overall survival was significantly shorter in the TD group (41.5 vs. 49.4 months; $P=.024$) and toxicity was higher with TD.

Lenalidomide

A phase III double-blind, randomized study compared MPR-R with MPR or MP followed by placebo in patients 65 years of age or older with newly diagnosed MM. Response rates were superior in both the lenalidomide-containing regimens. MPR-R significantly prolonged PFS (median, 31 months) as compared with MPR (median, 14 months) and MP (median, 13 months). No difference, however, was seen in OS.

Treatment of relapsed and refractory MM

Relapsed MM is defined as disease that progresses and requires salvage therapy. Relapsed/refractory MM is defined as disease that is nonresponsive to salvage therapy, or progresses within 60 days of last treatment in patients who previously achieved at least a minimal response (MR). These entities are distinguished from primary refractory MM, which refers to disease that fails to achieve at least an MR with initial therapy. These definitions are based on the EBMT criteria and IMWG uniform criteria.

The prognosis associated with relapsed MM is generally poor, with a median overall survival of <1 year among patients who have received two or more prior lines of therapy. Patients are refractory to bortezomib and relapsed following immunomodulatory drug have a median overall survival and EFS of 9 and 5 months, respectively.

The choice of therapy in the relapsed setting depends on the depth and duration of response to prior therapies, as well as treatment-related toxicities. Short duration of response and progression while on therapy are associated with adverse outcome. Other factors such as performance status, comorbid conditions, and goals of care are also critical in making decisions of therapy.

A patient who is naïve to an agent with known activity in MM typically is treated with a regimen incorporating this agent. Similarly, a patient with relapsed MM who previously has not undergone SCT can be considered for high-dose therapy, as well as patients who experienced prolonged response to first SCT. Retreatment of a patient with an effective agent can be performed at relapse with the same drug alone or in combination with other agents. Although a patient may have shown resistance to a specific drug at an earlier point in the disease, the agent may be employed at time of relapse in combination with other drugs with which synergy exists.

Specific agents used in relapsed/refractory MM

Immunomodulators in relapsed/refractory MM

Thalidomide

The activity of thalidomide was first demonstrated in a phase II trial by Singhal et al. (1999) in which 84 individuals with relapsed and refractory MM received thalidomide monotherapy at doses ranging from 200 to 800 mg/d. The ORR in this heavily pretreated population was 32%. The 2-year EFS and OS rates of 169 patients who ultimately enrolled onto this trial were 20% and 48%, respectively, with 10-year EFS and OS rates of 6% and 10%, respectively. These results were confirmed by other clinical trials involving thalidomide. A systematic review of 42 phase II trials involving 1,674 patients with relapsed and refractory MM showed that thalidomide monotherapy produces an ORR of 29% and a median OS of 14 months.

Lenalidomide

Two large, randomized, phase III clinical trials in relapsed MM—the MM-009 North American study and the MM-010 European/Israeli/Australian study—have been published. Study participants were randomized to either placebo or lenalidomide 25 mg on days 1 to 21 of each 28-day cycle. Dexamethasone 40 mg was administered to both treatment groups on days 1-4, 9-12, and 17-20 during the first four cycles and on days 1-4 only thereafter. Lenalidomide and dexamethasone produced superior ORRs in both MM-009 (61% vs. 19.9%) and MM-010 (60% vs. 24%). Median TTP, the primary endpoint of the trial, was significantly longer in both the MM-009 (11.1 vs. 4.7 months) and MM-010 (11.3 vs. 4.7 months) studies. Subgroup analysis of MM-009 and MM-010 demonstrated that, as compared with dexamethasone alone, lenalidomide plus dexamethasone conferred a benefit in ORR, TTP, and PFS regardless of prior thalidomide exposure.

The combination of lenalidomide, cyclophosphamide, and dexamethasone (LCD) regimen also can be utilized in

the relapsed setting. This combination was evaluated in a phase I/II study in which the ORR was 65%, the CR rate 5%, and the rate of VGPR or better was 15%.

Pomalidomide

Pomalidomide (Celgene, New Jersey) is a second-generation oral immunomodulatory drug that has demonstrated significant activity in MM in vitro and high response rates in patients refractory to other lines of treatment, including bortezomib and lenalidomide. Several clinical trials have been performed using pomalidomide alone or in combination with dexamethasone in patients with relapsed or refractory MM. In some trials, patients were refractory to lenalidomide and bortezomib. These trials demonstrated further evidence of the synergistic activity and efficacy of pomalidomide and dexamethasone. Pomalidomide, with or without dexamethasone, produces consistent and durable responses in patients with advanced disease who are refractory to multiple lines of therapy, including bortezomib and lenalidomide. They showed encouraging PFS and OS. The combination regimen is generally well tolerated and the most common adverse events were cytopenias. Pomalidomide currently is being investigated in phase III trials with other combination therapies, including bortezomib.

Most recently, the results of the IFM 2009-02, a phase II randomized trial of two different treatment schedules of pomalidomide and dexamethasone in patients refractory to multiple lines of therapy, including lenalidomide and bortezomib, were presented in the 2011 American Society of Hematology (ASH) meeting. Eighty-four patients were enrolled in the study with median number of previous therapies of five, with the highest previous lines of therapy of 13. One arm received pomalidomide at 4 mg from days 1-21 and the other received 4 mg from days 1-28 of each 28-day cycle along with 40 mg of oral dexamethasone weekly. Patients received therapy until disease progression and the median follow up was 11.3 months. The ORR was 35% in arm 21/28 and 34% in arm 28/28, including 4.7% and 7.3% having a VGPR or greater and 44% and 51% of patients having stable disease. The median duration of response was 10.5 months and 7.2 months in arm 21/28 and 28/28, respectively. The most common grade 3 and 4 AEs were cytopenias in 72% of patients, in particular, neutropenia. This agent is under consideration for U.S. Food and Drug Administration (FDA) approval for patients with relapsed MM.

Proteasome inhibitors in relapsed/refractory MM

Bortezomib

Phase I and II studies, involving individuals with relapsed MM, demonstrated manageable treatment-associated toxicity

and significant activity in this setting. These studies were followed by a phase III study in which 669 patients with relapsed MM were randomized to receive either bortezomib 1.3 mg/m² on days 1, 4, 8, and 11 of each 21-day cycle for eight 3-week cycles followed by treatment on days 1, 8, 15, and 22 for 5-week cycles or dexamethasone 40 mg on days 1-4, 9-12, and 17-20 for four 5-week cycles followed by treatment on days 1-4 for five 4-week cycles. Bortezomib was superior to high-dose dexamethasone with respect to ORR (38 vs. 18%), CR rate (6% vs. 1%), median TTP (6.22 vs. 3.49 months), and 1-year OS rate (80% vs. 66%). With extended follow-up of study participants, the ORR and CR rates among bortezomib-treated patients increased to 43% and 95%, respectively. The median OS was 29.8 months in the bortezomib arm versus 23.7 months in the dexamethasone arm.

The addition of anthracyclines to bortezomib has also shown modest efficacy in MM. In a phase III trial of 646 patients with relapsed MM, patients received either bortezomib 1.3 mg/m² on days 1, 4, 8, and 11 of each 21-day cycle or the same regimen of bortezomib in combination with pegylated liposomal doxorubicin 30 mg/m² on day 4. The combination was more superior to bortezomib alone in terms of median TTP (9.3 vs. 6.5 months) and 15-month OS (76% vs. 65%). Although grade 3 and 4 toxicities, such as anorexia, vomiting, thrombocytopenia, neutropenia, and hand-foot syndrome, occurred more frequently with the doublet, cardiac toxicity was only minimally increased with the combination, and rates of neuropathy were nearly equivalent.

Combinations of an immunomodulatory drug and bortezomib

High levels of response have been achieved with combinations of bortezomib and either thalidomide or lenalidomide. This is consistent with preclinical data demonstrating synergy between these drug classes. A phase I/II study of 85 patients receiving bortezomib in combination with thalidomide and dexamethasone (BzTD) yielded an ORR (MR or better) of 79%, a PR rate of 63%, and nCR rate of 22%. The 4-year EFS and OS were 6% and 23%, respectively. In another phase I/II study, the combination of lenalidomide, bortezomib, and dexamethasone was studied in patients with relapsed MM. The ORR (MR or better) was 86% and the CR/nCR rate was 24%. The most common grade 3/4 therapy-related toxicities were neutropenia, thrombocytopenia, anemia, and leukopenia.

Carfilzomib

Carfilzomib is a second-generation proteasome inhibitor that selectively and irreversibly binds to its target resulting in sustained inhibition. Carfilzomib has demonstrated durable

antitumor activity and an acceptable tolerability profile in patients with MM. It recently received FDA approval in patients with relapsed MM. An open-label, single-arm phase II study (PX-171-003-A1) was conducted in patients with relapsed MM. Patients received single-agent carfilzomib 20 mg/m² intravenously twice weekly for 3 of 4 weeks in cycle 1, then 27 mg/m² for </= 12 cycles. A total of 266 patients were evaluable for safety, 257 for efficacy; 95% were refractory to their last therapy; 80% were refractory or intolerant to both bortezomib and lenalidomide. Patients had median of five prior lines of therapy, including bortezomib, lenalidomide, and thalidomide. Overall response rate was 23.7% with median duration of response of 7.8 months. Median overall survival was 15.6 months. AEs were manageable without cumulative toxicities. Common AEs were fatigue (49%), anemia (46%), nausea (45%), and thrombocytopenia (39%). Thirty-three patients (12.4%) experienced peripheral neuropathy, primarily grades 1 or 2. Carfilzomib has less neuropathy compared with bortezomib. Further studies are ongoing using carfilzomib at higher doses or in combination with lenalidomide in the upfront or relapsed/refractory setting.

Other second-generation proteasome inhibitors such as MLN9708 (Millennium/Takeda, MA), Marizomib (NPI-0052, Nereus, CA), and Onyx 0912 (Onyx, CA) and are being tested in clinical trials in relapsed/refractory MM.

Other agents being studied in clinical trials in relapsed/refractory MM

Monoclonal antibody therapy is emerging as a new modality of therapy for patients with MM. Examples of such antibodies include the anti-CS1 monoclonal antibody elotuzumab and the anti-CD38 antibody that currently are being investigated. Recent results were presented in ASH 2011 for the phase II study of elotuzumab in combination with lenalidomide and dexamethasone in relapsed/refractory myeloma patients that were naïve to lenalidomide. Seventy-three patients were randomized to receive elotuzumab 10 mg/kg or 20 mg/kg intravenously (IV) weekly on a 28-day cycle for the first two cycles and days 1 and 15 of subsequent cycles, lenalidomide 25 mg orally days 1-21 and 40 mg oral dexamethasone weekly. Fifty-five percent of patients had received two or more prior therapies and 82% of patient had received ASCT. The overall response rate was 82%, including 26 patients achieving VGPR, and of those 9 patient achieved complete response. The ORR in the 10 mg/kg group was 92% and in the 20 mg/kg group was 73%. These results are encouraging for the further testing of monoclonal antibodies in patients with relapsed MM.

Signaling inhibitors, such as inhibitors of the PI3K/Akt pathway, also have been tested. These include perifosine, an alkylphospholipid that targets Akt, as well as mTOR inhibitors.

Perifosine has been administered in conjunction with dexamethasone, lenalidomide, and bortezomib and currently is being assessed in a phase III study of bortezomib and dexamethasone plus perifosine/placebo. Temsirolimus, an inhibitor of TORC1 has demonstrated significant activity in combination with bortezomib in relapsed MM.

The histone deacetylase (HDAC) inhibitors vorinostat, panobinostat, and romidepsin are being evaluated in relapsed MM. Although these agents have modest single-agent activity, they possess significant anti-MM effect in combination with agents, such as lenalidomide or bortezomib. HDAC inhibitors in combination with proteasome inhibitors have demonstrated synergistic activity in preclinical studies. This has been confirmed in phase I and II clinical trials with class I and II inhibitors in combination with vorinostat or panobinostat, in patients with relapsed and refractory MM. Vorinostat (Merck, MA) is an oral HDAC inhibitor, which blocks HDAC1, HDAC2, HDAC 3, and HDAC 6. It has been approved since 2006 in the treatment of cutaneous T-cell lymphoma. The results of the Vantage 088 trial were presented at ASH 2011, an international, multicenter phase III, randomized, double-blind study of vorinostat or placebo in combination with bortezomib in patients with relapsed MM. Six hundred and thirty-seven patients were randomized to therapy. The overall response rate was 56% in the vorinostat group compared with 41% in the placebo group ($P < .0001$). PFS was 7.63 months versus 6.83 months with a statistically significant reduction in the HR of 23%. There was not a statistical difference in OS. Gastrointestinal and hematologic toxicity were the most common AEs.

The list of novel agents is increasing rapidly as new targets are identified, and further investigation of these novel strategies, both alone and in combination, for the treatment of MM is warranted as part of a continued effort to improve patient outcome. Thus, participation in clinical trials is critical and a cornerstone of patient management in the relapsed or relapsed/refractory setting. Until recently, management strategies for patients with MM have been limited. With the introduction of novel targeted therapies and combinations with these and other agents, the opportunity to improve responses in this patient population has dramatically increased.

Supportive care

Several agents and treatments are important components of the supportive care for patients with MM. Bisphosphonates, such as pamidronate or zoledronic acid, reduce skeletal events in patients with lytic bony lesions, and monthly infusions of one of these agents are part of the standard care of patients with lytic lesions. Complications include osteonecrosis of the jaw, which, although rare, can be challenging.

Avoidance of invasive dentistry and prolonged use of inhibitors in cases in which infiltration occurs during bisphosphonate therapy are important considerations. Albuminuria secondary to pamidronate can be seen, as well as glomerulonephropathy with zoledronic acid. Treatment involves cessation of bisphosphonate, and the nephropathy usually is reversible. Bisphosphonates have an effect on survival of patients with MM came from a recent randomized study of the Medical Research Council (MRC) IX. This study showed a significant improvement of survival in patients who receive zolendronic acid, indicating that bisphosphonates do not only prevent lytic lesions but also can help control MM tumor growth. Denosumab, a fully human monoclonal antibody to RANKL, was developed to treat patients with skeletal diseases. It is approved for the treatment of cancer-related skeletal disease except for MM because of the lower survival rate that was observed in a large randomized study. Further clinical trials of this agent are ongoing in MM. In addition, a neutralizing anti-DKK1 inhibitor antibody (BHQ880, Novartis, NJ) was tested in vivo and demonstrated reduced osteolytic bone resorption, increased bone formation, and helped control MM growth in mice in vivo.

Patients with myeloma are subject to developing VTE. The risk of VTE is compounded by the use of IMiDs and corticosteroids. A prospective trial compared aspirin (ASA) to low-molecular weight heparin (LMWH) in 342 newly diagnosed patients who were treated with MPR. Patients were randomly assigned to receive ASA 100 mg/d ($n = 176$) or LMWH enoxaparin 40 mg/d ($n = 166$). The incidence of VTE was 2.27% in the ASA group and 1.20% in the LMWH group, a difference that was not significant. In the basis of these results, ASA can be an effective and less-expensive alternative to LMWH thromboprophylaxis. Of note, however, patients considered to be at high risk for VTE were excluded. Those patients with advanced age, inherited thrombophilic abnormalities, recent surgery, or a history of VTE should be considered for full-dose anticoagulation. Another phase III trial of 667 patients treated with thalidomide-based regimens showed that ASA and WARfarin had similar efficacy in reducing serious thromboembolic events, acute cardiovascular events, and sudden deaths compared with LMWH, except in elderly patients, in whom WAR showed less efficacy than LMWH. The IMWG recommends that patients treated on immunomodulatory agents should be assessed for risks of deep venous thrombosis; for those with no or one risk factor, aspirin 81–325 mg once daily is recommended, whereas those with two or more risk factors, LMWH (equivalent of enoxaparin 40 mg once daily) or full-dose warfarin, international normalized ratio (INR) 2–3, is recommended.

The role of plasma exchange in patients with acute renal failure at the onset of MM was assessed in a randomized study of 104 patients. Study participants were randomly

assigned to conventional therapy plus five to seven plasma exchanges of 50 mL/kg of body weight of 5% human serum albumin for 10 days or conventional therapy alone. There was no conclusive evidence that plasma exchanges substantially reduced a composite outcome of death, dialysis dependence, or glomerular filtration rate at 6 months. A randomized study of IVIG use in patients with myeloma was conducted in the United Kingdom and showed that IVIg reduced life-threatening infections and significantly reduced the risk of recurrent infections. Anemia is common in myeloma, due to direct marrow effects of the myeloma, renal insufficiency, or chemotherapy. Recombinant human erythropoietin (rhEPO) can improve both the hemoglobin level and quality of life in patients with MM.

Other elements of supportive care in MM relate to the specific complications of the disease. Radiation therapy can be useful for painful lytic lesions or spinal compression fractures. Orthopedic consultation is important for impending fractures in weight-bearing bones. Kyphoplasty is also as a useful modality in managing compression fractures of the spinal column. Hypercalcemia is treated primarily with intravenous fluids and bisphosphonates. The risk of renal dysfunction is reduced by maintaining hydration and by avoiding nephrotoxins such as intravenous contrast dye or aminoglycoside antibiotics and by judicious use of nonsteroidal anti-inflammatory drugs. Because of the hypogammaglobulinemia associated with the disease, patients are also at considerable risk of infection, particularly at later stages of the disease. Therefore, antibiotics are an important part of the supportive care of patients with advanced myeloma.

Plasmacytoma

Patients who present with an apparent solitary bone lesion should be staged carefully to eliminate the possibility of more generalized disease. The most common site is the vertebral column, followed by the pelvis, femur, and humerus. Many patients with solitary plasmacytoma of the bone have either no or low levels of M protein in serum or urine, and serum levels of normal Ig usually are preserved, but patients may have elevated serum FLCs. Marrow biopsies are negative for increased plasma cells. MRI of the spine is an important tool and may reveal abnormalities not previously detected by skeletal survey and thus may upstage up to a quarter of patients to MM. Local therapy for solitary plasmacytoma of bone consists of radiotherapy of 40–50 Gy. PET-computed tomography scan may be a useful staging modality in such patients. Patients with subsequent disappearance of any detectable M protein by immunofixation and no evidence of active disease have the longest stability and a chance of cure. Otherwise, the majority of patients progress to myeloma

within a median of 2 years; only 20% remain free of disease at 10 years.

A solitary extramedullary soft tissue plasmacytoma is more likely to be truly localized than a plasmacytoma of bone. The most common sites are in the upper respiratory tract (nasal cavity, sinuses, or pharyngeal lymph), but they can also occur in the gastrointestinal tract, central nervous system, urinary bladder, thyroid, breast, testes, parathyroid gland, or lymph nodes. Careful staging is necessary to rule out systemic disease. Because they are more likely to remain localized, there is a relatively high chance of cure with localized radiotherapy of 40–50 Gy; ~70% of patients will be alive and disease free at 10 years.

Plasma cell leukemia and extramedullary MM

Plasma cell leukemia (PCL) is a rare form of plasma cell dyscrasia (2%–4% of all MM). The World Health Organization (WHO) criterion for diagnosis of PCL is that plasma cells constitute >20% of cells in the peripheral blood with an absolute plasma cell count of more than $2 \times 10^9/L$. The presentation may be primary, de novo, or secondary, evolving from an existing case of MM as part of the terminal phase of the disease. The primary form accounts for 60% of the cases. In primary PCL, the constellation of adverse biologic prognostic factors in patients with advanced aggressive myeloma is already present at diagnosis. In fact, primary PCL has a more aggressive clinical presentation than MM, with a higher frequency of extramedullary involvement, anemia, thrombocytopenia, hypercalcemia, and renal failure. PCL differed from MM in the expression of CD56, CD9 HLA-DR, CD117, and CD20 antigens. Most cases of PCL studied in one report were diploid or hypodiploid, whereas most MM cases (57%) showed DNA hyperdiploidy. With the FISH technique, 12 of 13 PCL cases displayed the numeric aberrations, -13 (86%), +/−1 (57%), +18 (43%), and -X in women (25%), but they lacked several numeric aberrations usually found in MM such as +3, +6, +9, +11, and +15. PCL cases had a lower overall response to therapy than MM cases (38% vs. 63%, $P = .01332$). Among PCL patients, a trend for a worse response was observed in cases treated with melphalan and prednisone (MP) versus polychemotherapy. Overall survival was significantly worse in PCL versus MM patients (8 vs. 36 months, $P < .0001$), but it was significantly better in PCL patients treated with polychemotherapy versus MP (18 vs. 3 months, $P = .0137$).

EMD is defined as a clonal plasmacytic infiltrate at anatomic sites distant from the BM or adjacent soft tissue in a patient with underlying MM. The exact prevalence of EMD, however, has not been reproducibly described, with some series citing EMD in 6% of myeloma patients and others up to 20%. By contrast, EMD has been identified in a high

proportion of myeloma patients at the time of death, with 40% exhibiting evidence of plasma cell infiltration of the liver on autopsy analysis in one series. Underlying biological changes that lead to extramedullary dissemination are not well known. In many patients, TP53 mutation may be a feature of disease relapse, whether localized to the marrow or at extramedullary sites. Surface markers of MM cells present in EMD include downregulation of CD56 and increased expression of CD44. Extramedullary MM is a highly aggressive disease entity with poor response to many treatment modalities. In the Total Therapy 3 Trial, the presence of EMD at the time of diagnosis conferred significantly decreased overall and EFS for the study duration. There is paucity of data regarding the treatment responsiveness of extramedullary MM. In particular, small studies have suggested that thalidomide may be ineffective against previously treated MM that progresses to extramedullary soft-tissue plasmacytoma. Still other studies have described the efficacy of lenalidomide and bortezomib for EMD. Newer data suggest that patients with relapsed, refractory MM and EMD may benefit from a pomalidomide-based chemotherapeutic regimen, with response rates as high as 30%.

Other plasma cell disorders

Amyloidosis

Table 23-7 summarizes the characteristics of the next three types of plasma cell disorders. AL is an uncommon disorder in which proteins change conformation, aggregate, and form fibrils that infiltrate tissues leading to organ failure and death. The most frequent types are light chain (AL) derived from monoclonal B-cell disorders producing amyloidogenic Ig light chains and the hereditary and “senile systemic” (ATTR) variants from mutant and wild-type transthyretin (TTR). Diagnosis requires tissue biopsy. AL is more frequent and causes more organ disease than ATTR. Although both can cause cardiomyopathy and heart failure, AL progresses more quickly, so survival depends on timely diagnosis. Typing usually is based on clinical and laboratory findings with monoclonal gammopathy evaluation and, if indicated, TTR gene testing. Direct tissue typing is required when a patient has two potential amyloid-forming proteins. In coming years, widespread use of definitive proteomics will improve typing. Amyloid is an infiltrative fibrillar protein with light chain sequences for each patient identical to those of the light chains produced by the monoclonal plasma cells. Of patients with MM, 10% have evidence of concurrent AL. Serum and urine immunofixation studies reveal an M protein in 80% of patients, with λ-light chains more frequent than κ-light chains. AL should be suspected in patients with myeloma or a monoclonal gammopathy who show evidence

Table 23-7 Other plasma cell disorders.

Waldenström macroglobulinemia	IgM monoclonal gammopathy (regardless of the size of the M protein) with >10% bone marrow lymphoplasmacytic infiltration (usually intertrabecular) by small lymphocytes that exhibit plasmacytoid or plasma cell differentiation and a typical immunophenotype (eg, surface IgM, CD5, CD10, CD19, CD20, CD23) that satisfactorily excludes other lymphoproliferative disorders, including chronic lymphocytic leukemia and mantle cell lymphoma. IgM MGUS is defined as serum IgM monoclonal protein level <3 g/dL, bone marrow lymphoplasmacytic infiltration <10%, and no evidence of anemia, constitutional symptoms, hyperviscosity, lymphadenopathy, or hepatosplenomegaly. Smoldering Waldenström macroglobulinemia (also referred to as indolent or asymptomatic) is defined as serum IgM monoclonal protein level >3 g/dL and/or bone marrow lymphoplasmacytic infiltration >10% with no evidence of end-organ damage, such as anemia, constitutional symptoms, hyperviscosity, lymphadenopathy, or hepatosplenomegaly.
Amyloidosis (AL)	Presence of an amyloid-related systemic syndrome (such as renal, liver, heart, gastrointestinal tract, or peripheral nerve involvement) with positive amyloid staining by Congo red in any tissue (eg, fat aspirate, bone marrow, or organ biopsy); plus evidence that amyloid is light chain-related, established by direct examination of the amyloid (immunoperoxidase staining, direct sequencing); plus evidence of a monoclonal plasma cell proliferative disorder (serum or urine M protein, abnormal free light chain ratio, or clonal plasma cells in the bone marrow). Approximately 2%-3% of patients with amyloidosis will not meet the requirement for evidence of a monoclonal plasma cell disorder and the diagnosis must be made with caution in these patients. In such patients, molecular amyloid fibril typing, such as mass spectrometry or immunogold techniques, may be essential.
POEMS syndrome	Presence of a monoclonal plasma cell disorder, peripheral neuropathy, and at least one of the following features: osteosclerotic myeloma, Castleman disease, organomegaly, endocrinopathy (excluding diabetes mellitus or hypothyroidism), edema, typical skin changes, and papilledema. The absence of either osteosclerotic myeloma or Castleman disease should make the diagnosis suspect. Elevations in plasma or serum levels of vascular endothelial growth factor and thrombocytosis are common features of the syndrome and are helpful when the diagnosis is difficult.

Ig = immunoglobulin; MGUS = monoclonal gammopathy of undetermined significance.

Adapted with permission, from Rajkumar V, et al. Multiple myeloma: diagnosis and treatment. *Mayo Clin Proc*. 2005;80:1371-1382.

of unexplained fatigue or weight loss, cardiomyopathy, orthostatic hypotension, macroglossia, nephrotic syndrome, carpal tunnel syndrome, peripheral or autonomic neuropathy, periorbital purpura, or hepatomegaly.

Diagnosis is confirmed by the presence of apple-green birefringence on polarized light examination of a tissue biopsy stained with Congo red. Baseline workup, including echocardiogram or cardiac MRI, should be considered. Survival is usually poor but is shortest in those patients with cardiomyopathy, orthostatic hypotension, renal failure, hepatomegaly, cachexia, or overt MM. The major determinants of early mortality are the presence of cardiac involvement, lower serum albumin, higher serum creatinine, and a higher number of organs involved. Among 271 patients undergoing SCT, troponin T was a powerful predictor of treatment-related (28% vs. 7% for troponin >0.06). Patients with troponin T levels exceeding 0.06 microg/L should be considered for less toxic therapies.

Treatment of AL is similar to that for myeloma, although the amyloid deposits are generally irreversible, and an aim of therapy is to retard further amyloid deposition. Options of therapy include melphalan and dexamethasone, ASCT, and

novel therapies, including thalidomide, bortezomib, and lenalidomide. Hematologic CRs usually are durable and result in long-term survival and a variable degree of organ recovery. A randomized trial was done that compared melphalan and dexamethasone (mel/dex) to HDT/SCT. The trial showed superior survival in the mel/dex arm, largely due to an unexpectedly high mortality rate (24%) in the patients assigned to HDT/SCT. Many centers are now reporting mortality risk has improved in recent years and is now well below 10%. Patients with impaired renal function often experience increased toxicity with melphalan at 200 mg/m²; therefore, the dose should be attenuated to 140 mg/m² to minimize occurrence of renal failure requiring dialysis, the risk of which is 5%. Participation in clinical trials for patients with these rare diseases is critical to improve the outcome and survival.

POEMS syndrome

POEMS (polyneuropathy, organomegaly, endocrinopathy monoclonal gammopathy, and skin changes) syndrome is a plasma cell dyscrasia that typically presents with a sensorimotor

peripheral neuropathy. Other features include hyperpigmentation, hypertrichosis, thickened skin, papilledema, lymphadenopathy, peripheral edema, hepatomegaly, splenomegaly, and hypothyroidism. Patients with POEMS syndrome are younger (median age, 51 years) and have longer average survivals (median, 8 years) than patients with symptomatic myeloma. The clinical course is commonly one of progressive neuropathy, and in almost all cases, the Ig light chain type is λ . Bone lesions are characteristically osteosclerotic. Therapy for POEMS syndrome includes radiation therapy if sclerotic disease is localized and is similar to clinical myeloma if diffuse. The preferred approach to treatment is ASCT. Clinical improvement is seen in nearly all patients following HDM/SCT. Among 59 POEMS patients treated with ASCT at one institution, 14 patients have progressed with a PFS of 98% and 75% at 1 and 5 years, respectively. Other treatment approaches include steroid-based therapy, and lenalidomide, which with less neurotoxicity than thalidomide, is seen as a promising new agent in this setting. There are few data regarding the use of bortezomib, which usually is avoided due to its potential for neurotoxicity.

Lymphoplasmacytic lymphoma

Waldenstrom Macroglobulinemia (WM), termed lymphoplasmacytic lymphoma in the WHO classification, is an indolent lymphoid malignancy composed of mature plasmacytoid lymphocytes that produce monoclonal IgM. The disease affects predominantly older patients, who present with anemia, lymphadenopathy, purpura, splenomegaly, elevated serum viscosity, neurologic signs and symptoms, or combinations of these findings. Lytic bone lesions typically are absent. The lymphoma cells may express a variety of markers, including CD5, CD19, CD20, and CD38, and surface or cytoplasmic Ig. Symptoms may be due to tumor infiltration (marrow, spleen, or lymph nodes), circulating IgM macroglobulin (hyperviscosity, cryoglobulinemia, or cold agglutinin hemolytic anemia), and tissue deposition of IgM or other proteins (neuropathy, glomerular disease, or amyloid).

Asymptomatic patients should be observed. Patients with a disease-related hemoglobin level <10g/L, platelet count <100 \times 10⁹/L, bulky adenopathy or organomegaly, symptomatic hyperviscosity, peripheral neuropathy, AL, cryoglobulinemia, cold agglutinin disease, or evidence of disease transformation should be considered for therapy. Plasmaapheresis should be considered for symptomatic hyperviscosity. Options of therapy for newly diagnosed patients with WM include the use of rituximab as monotherapy or in combination with cyclophosphamide, nucleoside analog, bortezomib, or thalidomide. Similar options can be used in the salvage setting. Newer agents, such as everolimus, also can be considered in the treatment of relapsed WM. Time to

response after rituximab usually exceeds 3 months on average. In a significant proportion of patients, a transient increase of serum IgM may occur immediately after initiation of rituximab (rituximab flare). This may lead to hyperviscosity in some patients. The combination of bortezomib and rituximab with or without dexamethasone has shown significant activity in the upfront or relapsed setting in WM. There is a high rate of peripheral neuropathy with the use bortezomib at the usual dosing schedule of twice a week. Therefore, the use of once-a-week regimens with bortezomib has showed high activity with less neuropathy. HDC with autologous stem cell rescue in primary refractory or relapsed disease should be considered for eligible patients. Allogeneic and “nonmyeloablative allogeneic” transplantations, however, should be approached cautiously, given the associated high mortality or morbidity risks, and should be undertaken only in the context of a clinical trial. Many novel therapeutic agents are being tested in clinical trials for WM. Given the paucity of large clinical trials in WM, especially phase III clinical trials, the establishment of a standard treatment regimen that can be used for the comparison of the response obtained with these clinical trials is challenging.

Key points

- MGUS is present in approximately 3% of individuals >70 years of age.
- The CRAB criteria are used to distinguish between MGUS and SMM and symptomatic MM. Only symptomatic MM requires therapy.
- MM may be treated with a variety of therapeutic regimens, including SCT. Thalidomide and derivatives, including lenalidomide, as well as other novel agents, such as bortezomib, which is a first-in-class proteasome inhibitor, are promising new approaches that now offer a significantly more positive outlook for patients with this otherwise incurable malignancy.
- Bisphosphonates are an important component of supportive care to decrease skeletal complications in myeloma.
- AL may be suspected in myeloma patients with cardiomyopathy, peripheral neuropathy, nephrotic syndrome, and macroglossia.
- WM presents clinically as an indolent B-cell lymphoma and may respond to nucleoside analogs, combination chemotherapy, or rituximab; promising investigational advances are also being made using combinations with novel agents.

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